

**Arizona Game and Fish Department Heritage Grant**  
***“Mycoplasma agassizii in Desert Tortoises”***  
**Project #U03005**

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## ABSTRACT

Upper Respiratory Tract Disease (URTD), caused by the pathogen *Mycoplasma agassizii*, poses a critical threat to the Mojave population of the desert tortoise (*Gopherus agassizii*). Release of captive tortoises into the wild has been implicated in the spread of the disease in the Mojave. However, little is known about URTD in the Sonoran population of the desert tortoise. To determine the distribution of URTD in Greater Tucson, Arizona we used enzyme-linked immunosorbent assay (ELISA) to detect antibodies indicating previous exposure to *M. agassizii* and polymerase chain reaction (PCR) to detect *M. agassizii* antigens indicating a current infection.

We collected blood and nasal lavage samples from 70 captive tortoises within Tucson and 138 free-ranging tortoises from 13 sites in 9 mountain ranges around Tucson, to compare results from 1) captive, 2) high-visitor impact, 3) suburban, and 4) remote populations to determine if there is an association between urbanization and distribution of *Mycoplasma agassizii*. We used radio-telemetry to determine home range sizes for tortoises at two sites in the Rincon Mountains, and then examined the association between ELISA results and home range size. We also determined the length of the active season for individual tortoises and examined the association between ELISA results and active season length.

*M. agassizii* antibodies varied by tortoise site category, with a higher percentage of ELISA positive tortoises in suburban areas than remote areas ( $p = 0.03$ ). Captive tortoises had a lower percentage of ELISA positive results than suburban tortoises ( $p = 0.04$ ). ELISA results also varied by age class, with the percentage of juvenile tortoises testing negative for *M. agassizii* significantly higher than adult tortoises ( $p = 0.0001$ ). We found no significant difference between ELISA positive and negative tortoises for 100% MCP ( $p = 0.89$ ), 95% kernel ( $p = 0.35$ ), or 50% kernel ( $p = 0.59$ ) home range size estimates. There was no significant difference in mean length of active season between ELISA positive and negative tortoises ( $p = 0.63$ ).

Our data indicate that captive tortoises in the Tucson area do not serve as an important reservoir for URTD. However, disease incidence was highest in suburban areas, suggesting that urbanization has a negative impact on tortoise health. Additional studies are necessary to evaluate the mechanisms by which urbanization may affect desert tortoise disease, movement, thermal ecology, and survival.

## INTRODUCTION

Over the past 25 years, the desert tortoise (*Gopherus agassizii*), the largest reptile native to the deserts of the southwestern United States, has experienced dramatic declines in some populations in the Mojave and Colorado Deserts (USFWS 1994, Berry 1997). These declines have been attributed to the cumulative impacts of human intervention, predation, habitat loss and degradation, and disease (USFWS 1994). In 1988, desert tortoises with Upper Respiratory Tract Disease (URTD) were found in the Desert Tortoise Natural Area (DTNA), Kern County, California (Jacobson et al. 1991). In 1989, a detailed survey of the DTNA and nearby areas indicated that 43% of 468 desert tortoises showed clinical signs of this disease (Knowles 1989). Six hundred twenty-seven carcasses were recovered from the DTNA during the survey (Knowles 1989). Between 1979-1992, the adult desert tortoise population declined 90% within the DTNA (Berry 1997). URTD was identified as a causative agent that led to this catastrophic decline.

Largely because of this disease, the Mojave population of the desert tortoise (north and west of the Colorado River) was granted an emergency designation as endangered under the Endangered Species Act in 1989 (USFWS 1989). Following subsequent surveys, the population was reclassified as threatened (USFWS 1990, 1994). In addition to the DTNA, desert tortoises with URTD have been documented in Las Vegas Valley, Nevada; the Beaver Dam Slope (along the Utah-Arizona border), and Yucca Mountain, Nevada (Jacobson 1993; Lederle et al. 1997; Dickinson et al. 2002). URTD has also been documented in the Arizona Sonoran desert tortoise population, (Barrett 1990a, b; Riedle and Averill-Murray 2003) though not in epidemic proportions (AIDTT 1996a, 2000; Dickinson, et al. 2002).

There is evidence that escaped or released captive desert tortoises pose a threat to healthy free-ranging populations as disease vectors. Clinical signs of URTD have been observed in captive tortoises for many years in California (Fowler 1976; Roskopf et al. 1981; Jacobson et al. 1991; Jacobson 1993; Berry et al. 2003). The highest prevalence of URTD in free-ranging tortoises in California was found at two study sites where previous releases of captive desert tortoises occurred (Jacobson et al. 1995). Ill captive tortoises are commonly returned to the wild due to the anxiety they generate in their custodians (Jacobson et al. 1995). A higher prevalence of URTD has been reported near urban areas, which often have high concentrations of captive desert tortoises (USFWS 1994).

No study has examined the interaction between captive and wild tortoises in the Tucson area, but thousands of tortoises have been held in captivity adjacent to some of the highest density wild populations reported in Arizona (Averill-Murray and Klug 2000). Since 1981, the Arizona-Sonora Desert Museum's (ASDM) Tortoise Adoption Program (TAP), which is sanctioned by the Arizona Game and Fish Department, has adopted more than 2,000 tortoises into approved homes in the Tucson area. In addition, non-native tortoises, which are commonly kept as pets, pose a threat as a potential vector of disease to free-ranging tortoises (J. L. Jarchow, DVM, personal communication). On two separate occasions in 2000, African spurred tortoises were removed from Gates Pass, in the Tucson Mountains (S. Poulin, ASDM, personal communication 2000). Both had only native vegetation in their fecal samples, which indicates that they had been living in that area for some time (M. Demlong, AGFD, personal communication 2001).

In 1994, *Mycoplasma agassizii* was found to be a causative agent of URTD in desert tortoises (Brown et al. 1994). Clinical signs of URTD include intermittent serous, mucoid, or purulent nasal discharge, ocular discharge, palpebral edema, conjunctivitis, eyes recessed into the orbits, and dullness of the skin and scutes (Jacobson et al. 1991; Schumacher et al. 1993; Brown et al. 1994). This disease is highly contagious and transmitted by close contact between tortoises. *Mycoplasma* infections are often clinically silent and long-lasting; some tortoises have remained infected for up to a year (McLaughlin 2000).

Monitoring the disease status of desert tortoises throughout their range is considered important for understanding the dynamics of URTD in wild populations (USFWS 1994). Little pathological information exists on either the captive or free-ranging Sonoran desert tortoise population (Jacobson et al. 1991; Dickinson et al. 2002). During 1991-1994, a health study on free-ranging Sonoran tortoises was conducted in the Harcuvar Mountains and Little Shipp Wash (Dickinson et al. 2002). Although no clinical signs of URTD were observed in these remote populations, three out of 99 tortoises (two in the Harcuvars and one at Little Shipp Wash) tested positive for *M. agassizii* antibodies or antigens.

More recently, a preliminary disease study was conducted during 2001-2002 at desert tortoise study sites in Arizona. While no *M. agassizii* antibodies were detected in tortoises at

three remote sites (Sugarloaf, Florence, and Silverbell Mountains), 23 out of 43 tortoises in two sites adjacent to Tucson (Saguaro National Park East (SNPE) and Ragged Top Mountain) tested positive for *M. agassizii* antibodies (Riedle and Averill-Murray 2003). None of the SNPE tortoises were exhibiting clinical signs of URTD at the time sampling occurred, but five tortoises at SNPE have exhibited clinical signs of URTD since 1999. These signs have included wheezing, wet and bubbling nasal discharge, and runny eyes. Observational records since 1999 indicate that these symptoms have sporadically recurred in all tortoises that have exhibited them (T. Esque and C. Schwalbe, U.S. Geological Survey, and D. Swann, National Park Service, unpublished data).

In our study, we examined URTD in desert tortoises in the vicinity of Tucson, Arizona. By comparing the ELISA and PCR analysis of the captive tortoises with results from free-ranging tortoises in high-visitor impact, suburban, and remote areas, we documented the distribution of *M. agassizii* in the Greater Tucson area. In addition, we compared home range size estimates of tortoises with and without URTD and examined thermal ecology in a population located in the Rincon Mountains using radio-telemetry and temperature-sensing dataloggers. This baseline data will be invaluable for monitoring the health of Tucson area desert tortoises over time, managing captive tortoises and adoption programs, and for developing conservation programs for the desert tortoise.

## OBJECTIVES

1. Determine the distribution of *Mycoplasma agassizii* in captive Sonoran desert tortoises in Tucson, Arizona.
2. Determine if there is a correlation between urbanization and distribution of *Mycoplasma agassizii* in wild populations of Sonoran Desert tortoises along an urban gradient in and around Tucson, Arizona.
3. Compare behavior, specifically thermal ecology and movement, and survival of Sonoran Desert tortoises that exhibit clinical signs of URTD with tortoises that do not exhibit clinical signs using radio-telemetry and temperature-sensing dataloggers.
4. Develop an educational outreach program press release and brochure to inform the public about laws, regulations, and biological impact regarding collecting and releasing desert tortoises.

## HYPOTHESES

We tested the hypothesis that the percentage of Sonoran desert tortoises that test positive for *Mycoplasma agassizii* will differ along an urban gradient with the most urbanized populations exhibiting the highest distribution. The null hypothesis is that there is no relationship between distribution of *M. agassizii* and location in Sonoran population of the desert tortoises. We also tested the hypothesis that *M. agassizii* affects individual tortoise home range size, thermal ecology, and survival. The null hypothesis is that there is no effect on these variables.

## METHODS

### **Tortoise Sample Groups.**

#### *Captive Tortoises.*

The Arizona-Sonora Desert Museum Tortoise Adoption Program (TAP) and members of the Tucson Herpetological Society facilitated access to captive Sonoran Desert tortoises from metropolitan Tucson. We compiled a list of 321 names and address of individuals that adopted desert tortoises between 1996-2002, then randomly selected two groups of 100 to receive a letter (Appendix A) requesting their participation in this study. The first 100 letters were mailed on 21 May 2003, a letter was mailed to the second 100 custodians on 5 Aug 03. All custodians who contacted us to participate were included in this study.

#### *Free-ranging Tortoises.*

Free-ranging desert tortoises were sampled from three groups along an urban gradient. The groups consisted of 1) high-visitor impact, 2) suburban, and 3) remote sites. High-visitor impact areas are very easily accessed; popular with recreationists; have paved parking lots, multiple-use trails, and trams / paved tours; and have public programs to educate visitors about desert tortoises in the area. Suburban areas have limited access to hiking trails, are bordered by development that has been established for more than five years, and desert tortoises are known to occur. Remote areas lack easy public access, are reached by traveling on primitive dirt roads, and some are behind locked gates.

### **Study Sites.**

We sampled captive desert tortoises from 49 residences in cities, towns, and communities in the Greater Tucson area (Catalina, Catalina Foothills, Green Valley, Marana, Sahuarita, Oro Valley, Tucson, and Vail), and free-ranging desert tortoises from four suburban (Rocking K Ranch, Rincon Mountains; Saguaro Ranch, Tortolita Mountains; Panther Peak Wash, Tucson Mountains, Saguaro National Park West (SNPW); and Tumamoc Hill), three high-visitor impact (Mother's Day Fire, SNPE; Visitor Center, SNPW; and Sabino Canyon Recreation Area, Santa Catalina Mountains), and six remote (Black Mountain, Desert Peak, Ninetysix Hills, Chiminea Creek, Rincon Mountains, SNPE; Stevens Mountain, Sierrita Mountains; and Derrio Canyon, Tortolita Mountains) sites in Pima and Pinal counties in the Greater Tucson Area (Figure 1).

### **Field Methods.**

We hand-captured and processed free-ranging tortoises July-October 2002 and 2003, and July-August 2004 using standard methods (Murray and Schwalbe 1997) following Arizona Interagency Desert Tortoise Team (AIDTT 2000) guidelines. With the exception of two occasions, we processed tortoises at the site of capture. On the two occasions, one at Black Mountain and one at Sabino Canyon, a volunteer carried a tortoise from its capture site to a central field processing location then returned it to the point of capture within 2 hours. These tortoises were transferred in a clean, cloth bags that were moistened to maintain temperature in between processing and during transportation. Bags were washed and disinfected before reuse. We used hand-held Global Positioning System (GPS) units (Garmin E-map, GPS III-plus, Geko201, Olathe, KS) to determine the location of each tortoise encountered as Universal Transverse Mercators (UTM's), with CONUS NAD 27 as the datum.

Unmarked tortoises were marked using the notching system previously used at each site (R. Averill-Murray, personal communication 2002), with new numbers following those from the previous studies. At sites without an established numbering system, we marked tortoises using the standard notching system for Arizona (AIDTT 2000). In addition to the notches, we also marked each tortoise with a small epoxy-covered number on the fifth vertebral scute for easy identification if recaptured (Appendix B).

#### *Physical Exam.*

We examined each tortoise for clinical signs of URTD (nasal discharge, ocular discharge, palpebral edema, and conjunctivitis), shell anomalies, and parasites, and to determine sex (Murray and Schwalbe 1997). When possible, we also looked for clinical signs of herpesvirus (presence of plaque or open sores in the mouth). We weighed tortoises with a 1, 5, or 10-kg spring scale and measured their Midline Carapace Length (MCL) with pottery calipers to the nearest 1 mm (Christopher et al. 1997). When possible, we documented incidences of harassment, injury, or predation by wild or domestic canids and felids on tortoises (Bjurlin and Bissonette 2001, A. Demnon personal communication 2004) and evidence of released captive tortoises (i.e. those with paint on their carapace or a hole drilled in the marginal scutes) by taking slide or digital photographs (Bjurlin and Bissonette 2001). We also took photographs of the carapace, plastron, and nares of each tortoise, which will be archived for future research (Berry 1990). To prevent the transfer of pathogens between tortoises, we wore fresh exam gloves for each tortoise and washed our hands and all equipment with veterinary disinfectant (Chlorhexidine diacetate; AIDTT 1996b) after processing each tortoise.

#### *Blood and Nasal Lavage Sampling.*

We collected blood from each tortoise by brachial or jugular venipuncture in order to run an enzyme-linked immunosorbent assay (ELISA) to detect antibodies indicating previous exposure to *M. agassizii*. We manually restrained each tortoise on a pedestal (inverted coffee can) to immobilize them during processing. We cleansed the area where blood was to be sampled with diluted betadine followed by rinsing with an alcohol swab (Berry and Goodlett 2000). We collected less than 1-cc of blood with a syringe and 25<sup>5</sup>/<sub>8</sub> gauge needle for ELISA analysis, and then applied pressure to the puncture site to prevent bleeding. Blood samples were immediately injected into a labeled lithium heparin Microtainer™ tube (Fischer Scientific, Pittsburgh, Pennsylvania), inverted gently 10-15 times, and placed on ice (Jacobson et al 1992) to prevent clotting. We centrifuged blood samples within 12 hours to separate the plasma. Plasma was transferred into a labeled Cryule® vial (#66021-750 VWR, West Chester, Pennsylvania), and stored at -20 °C in a manual defrost freezer. The red blood cells were archived for a future genetic study.

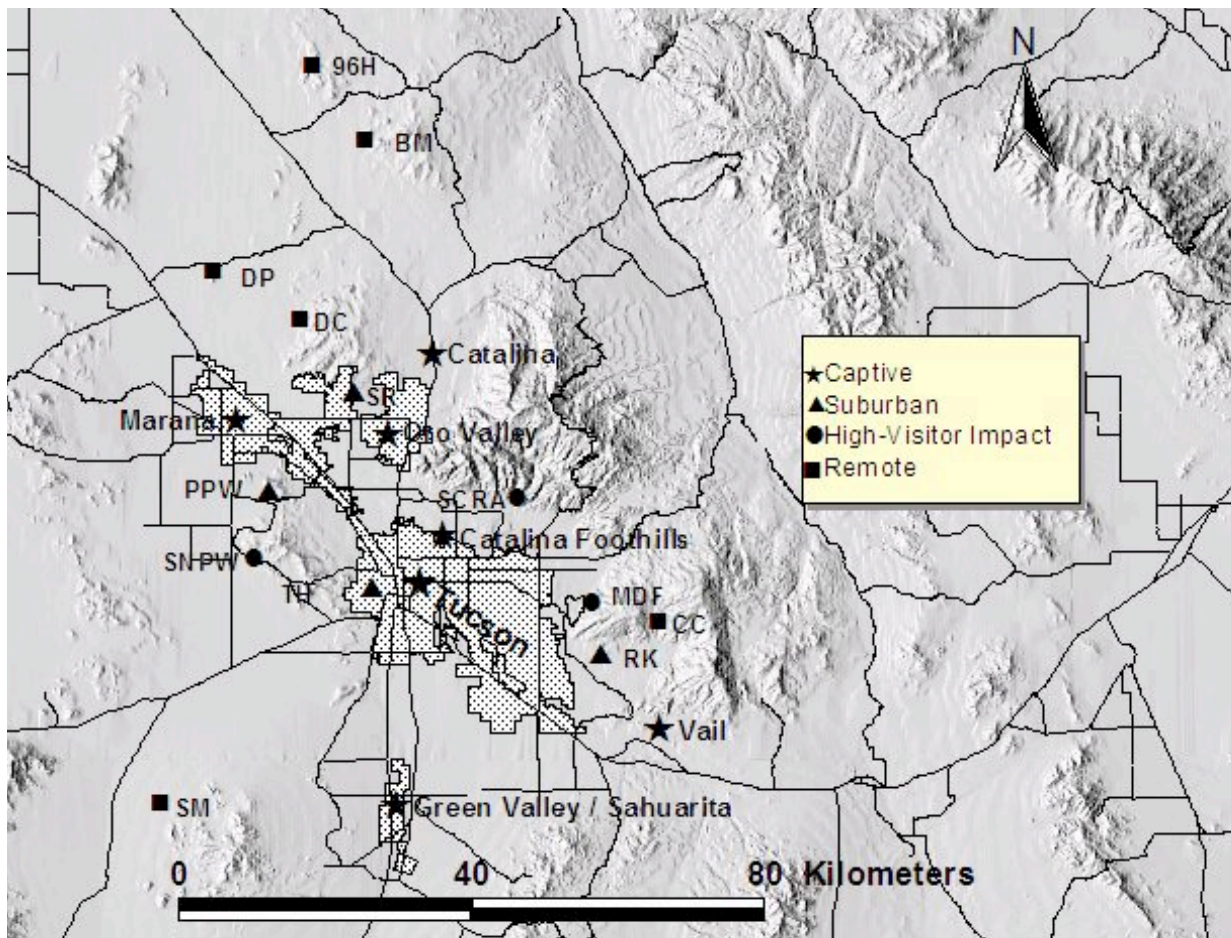
In August 2004, we collected a second blood sample from seven telemetered tortoises to determine if the ELISA titer level for *M. agassizii* changed during the study.

We performed a nasal lavage on each tortoise except when severe force would have been required to access the nare. The lavage samples were collected for PCR analysis. PCR results were used to determine if the tortoise was currently infected with *M. agassizii* (Brown et al. 1994; Brown et al. 1995). Prior to collecting the nasal lavage, we wiped off nares, face, and chin with an alcohol-soaked cotton ball, and allowed the skin air dry. We drew 3-cc of sterile 0.9% NaCl into a syringe, removed the needle, placed the open end of the syringe against each naris, then injected and aspirated each naris with 1.5-cc 0.9% NaCl (L. Wendland, Mycoplasma

Research Lab, University of Florida, personal communication). The nasal aspirate was transferred into a labeled 5-cc Corning® vial (#29442-542, VWR, West Chester, Pennsylvania), placed on ice, then transferred to a -20 °C manual defrost freezer as soon as possible.

#### *Rehydration and Release.*

We were prepared to rehydrate tortoises with a saline solution injection if they had an unusually low mass for body length, exhibited critical clinical signs of URTD, or voided excessively. We released each tortoise at the point of capture within 2 hours (AIDTT 1996b).



**Figure 1.** Location of sample sites for captive and free-ranging tortoises (with number sampled) from 13 populations in Greater Tucson, Arizona; Captive: Catalina (2), Catalina Foothills (12), Green Valley and Sahuarita (4), Marana (13), Oro Valley (5), Tucson (31), Vail (3); Suburban: Panther Peak Wash, SNPW, Tucson Mountains (PPW, 19), Rocking K Ranch, Rincon Mountains (RK, 18), Saguaro Ranch, Tortolita Mountains (SR, 4), Tumamoc Hill (TH, 8); High-Visitor Impact: Mother's Day Fire, SNPE, Rincon Mountains (SNPE, 25), Sabino Canyon Recreation Area, Santa Catalina Mountains (SCRA, 9), Visitor Center, SNPW, Tucson Mountains, (SNPW, 4); Remote: Ninetysix Hills (96H, 13), Black Mountain (BM, 17), Chiminea Creek, SNPE, Rincon Mountains (CC, 8), Derrio Canyon, Tortolita Mountains (DC, 4), Desert Peak (DP, 1), Stevens Mountain, Sierrita Mountains (SM, 9).

### **Diagnostic Tests.**

The Mycoplasma Research Laboratory, College of Veterinary Medicine, University of Florida (Gainesville) performed the ELISA and PCR diagnostic tests. We shipped the samples overnight on dry ice to the lab at the end of each field season.

#### *ELISA.*

An ELISA is currently the most effective, rapid, and inexpensive way to detect the specific antibody in plasma or serum that would be present after exposure to *M. agassizii* (Schumacher et al. 1993). A positive result indicates that the tortoise has been previously exposed to *M. agassizii*. A negative result indicates that there are no detectable antibodies to *M. agassizii* in the plasma provided to the laboratory. A negative result does not mean that the tortoise will never develop the disease; it indicates that there are no antibodies present at the time the blood sample was taken. A suspect result indicates that the antibody level is intermediate between positive and negative, and is considered inconclusive.

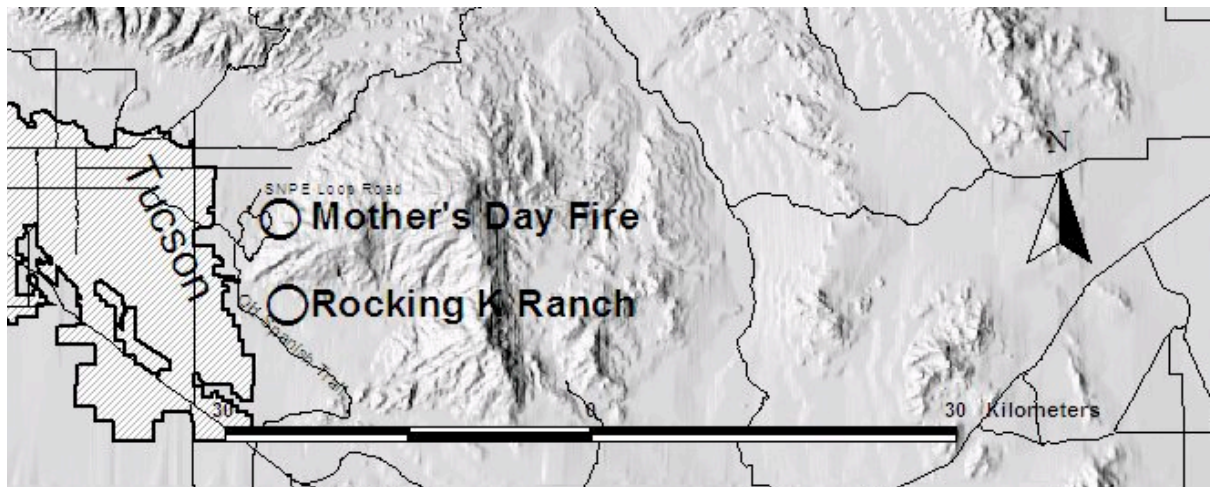
Clinical signs may appear within one or two weeks post-exposure, but it takes six to eight weeks for an exposed tortoise to develop antibodies detectable by an ELISA (McLaughlin 2000; Diemer Berish 2000). ELISA values are expressed as titers between the optical density of the plasma sample and that of a negative control. Sample titers <32 are negative, titers between 32-64 are suspect, and titers >64 are positive for *M. agassizii* (Schumacher et al. 1993). However, this serologic technique only indicates that a tortoise has been exposed and immunologically reacted to *M. agassizii* and, therefore, cannot distinguish between asymptomatic carriers (which pose a threat to healthy tortoises) and tortoises that have cleared the pathogen and are no longer infected (Brown et al. 1994; Schumacher et al 1997).

#### *PCR.*

PCR analysis is designed to detect *Mycoplasma* ribosomal ribonucleic acid (rRNA) gene sequences in nasal secretions of desert tortoises (Brown et al. 1995). This technique, often used in conjunction with ELISA, detects the *M. agassizii* antigen. A positive result indicates that the tortoise is currently infected with *M. agassizii*. A negative result indicates the tortoise is not currently infected with the *M. agassizii*, or the *Mycoplasma* bacteria are in numbers too low to be detected by PCR.

### **Radio-telemetry.**

We used radio-telemetry to track desert tortoises from May 2002–November 2004 at two sites in the Rincon Mountains (Figure 2). This radio-telemetry study included 17 tortoises that tested seropositive and four that tested seronegative for *M. agassizii* in 2002-2004, although not all of these tortoises were part of the study at the same time. The first site (Mother's Day Fire) lies entirely within the Saguaro National Park East (SNPE) boundary; telemetry data from 5-15 tortoises has been collected from this site since 1997 to determine the response of desert tortoises to fire (Esque et al. 2002, Esque et al. 2003) and for a reproduction study (Stitt 2004). We tracked 10 seropositive and 2 seronegative tortoises at this site. The second site (Rocking K) is approximately 6.5 km south of the Mother's Day Fire site (Figure 2) and is located along the SNPE south boundary at the Rocking K Ranch. Twenty-five tortoises have been radio-tracked at this site since July 1999 to collect data for studies on reproduction (Stitt 2004) and the response of desert tortoises to urban development (Swann et al. 2001, Swann et. al 2002). We tracked 7 seropositive and 2 seronegative tortoises at this site.



**Figure 2.** Radio-telemetry study sites at Mother's Day Fire (within SNPE) and Rocking K Ranch (adjacent to SNPE) in the Rincon Mountains.

We affixed transmitters (AVM Instruments Co., Livermore, California) to the right front side of the carapace using quick-drying epoxy, with the antenna threaded through rubber tubing to facilitate transmitter replacement (Boarman et al. 1998). Care was taken to not epoxy across scute seams where shell growth occurs.

Tortoises were tracked once every 7-10 days during the active season (March-October) and every other week during the inactive season (November-February) using a directional antenna and receiver. Each time a tortoise was located during radio-telemetry, its location was recorded with a GPS unit.

### **Thermal Ecology.**

We affixed iButtons (Thermochron DS1921G iButton, Maxim Integrated Products, Inc., Sunnyvale, CA), which are small temperature data loggers (4-g), to telemetered tortoises at the Mother's Day Fire site in November 2002, and to telemetered tortoises at both the Mother's Day Fire and Rocking K Ranch sites in November 2003 to collect data on environmental temperature selection during over-winter hibernation. Dataloggers were programmed to record temperature data every 2 hours, and then glued to the left rear carapace using quick dry epoxy. In December of 2002 and 2003, we also affixed dataloggers to rocks using epoxy and placed them in over-winter burrows of tortoises equipped with iButtons to collect burrow temperatures. We removed dataloggers from tortoises after they emerged from over-winter hibernation, and downloaded the information using iButton-TMEX (32-Bit) V3.20 (Dallas Semiconductor, Dallas, Texas).

### **Data Analyses.**

We performed all statistical analyses with JMP software (Ver. 4.0; SAS Institute, Inc.). We used chi-square tests to determine if there was a difference between ELISA results among the four groups of tortoises. We used logistic regression to examine if there were differences between the presence of positive ELISA results and clinical signs (Zar 1996).

We estimated home range size of radio-telemetered tortoises with 100% minimum convex polygon (White and Garrott 1990) and 50% and 95% fixed kernel methods (Worton 1989), using the Animal Movement Extension (Hooge et al, 1999) in ArcView GIS 3.3 (ESRI; Redlands, California). Home range size estimates are for the 2002-2004 active seasons. We examined the association between disease status and sex on home range size in the active season

using multiple regression (general linear model), with 100% MCP, 95% kernel, and 50% kernel as response variables, ELISA results and sex as explanatory variables, and number of observations in each active season as a covariate (Zar 1996).

We examined the association between disease status and sex on length of the active season using multiple regression (general linear model), with total number of days active in 2002-2004 as the response variable, ELISA results and sex as explanatory variables, and year as a covariate (Zar 1996). The active season for desert tortoises is generally March–October (Averill-Murray et al. 2002). We tailored the active season length for our telemetered tortoises using observation data recorded when tortoises were located. We defined the active season starting on the first date the tortoise was alert in its hibernacula or the tortoise had moved more than 10 m, and ending when the tortoise was no longer alert in its hibernacula or had moved less than 5 m in two consecutive observations, while taking into consideration the typical desert tortoise active season. Based on our tracking schedule, we assumed the total error in any given length of tortoise active season to be 14-20 days.

We determined the mean daily environmental temperature experienced by tortoises and the number of basking days for each tortoise using the datalogger data collected from December 2002 – March 2003. A basking day was defined as a day in which the tortoise temperature was  $\geq 5^{\circ}\text{F}$  above the burrow temperature for at least two consecutive measurements (measurements were recorded once every two hours).

## RESULTS

### **Tortoise Sample Results.**

Thirty-five desert tortoise custodians responded to the first letter and set appointments, yielding 36 samples; 11 letters were returned with no forwarding information. Thirty-two custodians responded to the second letter and set appointments, yielding 25 samples; 12 letters were returned without forwarding information. Nine desert tortoises in the holding pens at the Arizona-Sonora Desert Museum were sampled prior to adoption. A total of 70 captive desert tortoises from 49 residences in eight communities were included in this study (Figure 1, Table 1).

We sampled 49 tortoises from suburban sites, 38 tortoises from high-visitor impact sites, and 51 tortoises from remote sites, for a total of 138 free-ranging tortoises from 13 sites (Figure 1, Table 1).

In 2002, tortoises that had been captured previously did not test positive for *Mycoplasma* antibodies more frequently than tortoises captured for the first time (Pearson chi-square = 0.81,  $p = 0.37$ ,  $df = 1$ ), indicating no significant research effect, so we subsequently sampled both previously marked and unmarked tortoises from each site. At the conclusion of this study, we also found no significant research effect (Pearson chi-square = 2.36,  $p = 0.12$ ,  $df = 1$ ) between previously captured and newly captured tortoises.

### **ELISA Results.**

We collected 208 blood samples from tortoises in 227 attempts, and 205 nasal flush samples in 208 attempts. Tortoises sampled ranged in length from 131-303 mm MCL (mean = 231 mm,  $n = 194$ , 95% CI = 226 to 236 mm), and in mass from 370-6300 g (mean = 2300 g,  $n = 194$ , 95% CI = 2170 to 2431 g).

Of the 188 adult tortoises tested for *M. agassizii* antibodies using ELISA, 100 (53.2%) were seropositive, 70 (37.2%) were seronegative and 18 (9.6%) were suspect. Of the 114 males,

64 (56.1%) were seropositive, 40 (35.1%) were seronegative, and 10 (8.8%) were suspect. Of the 74 females, 36 (48.7%) were seropositive, 30 (40.5%) were seronegative, and 8 (10.8%) were suspect. Of the 20 juveniles, 0 were seropositive, 15 (71.4%) were seronegative, and 6 (28.6%) were suspect.

Of the 122 adult free-ranging desert tortoises sampled, 69 (56.6%) were seropositive, 42 (34.4%) were seronegative, and 11 (9.0%) were suspect. All 16 (100%) juvenile free-ranging desert tortoises were seronegative. Of the 64 adult captive tortoises sampled, 31 (48.4%) were seropositive, 26 (40.6%) were seronegative, and 7 (10.9%) were suspect. Two (34.6%) of the seven juvenile captive desert tortoises were seronegative, 5 (71.4%) were suspect.

**Table 1.** Locality names and number of tortoises sampled from each site in all four categories, with number of tortoises with positive clinical sign (CS +) and ELISA (ELISA +) results. Captive sites include number of residences sampled in each city, town, or community in Greater Tucson.

<b>Captive Sites in Greater Tucson</b>	<b># Samples</b>	<b># CS +</b>	<b># ELISA +</b>
Catalina (1)	2	1	2
Catalina Foothills (7)	12	0	7
Green Valley, Sahuarita (3)	4	0	2
Marana (9)	13	0	7
Oro Valley (4)	5	1	2
Tucson (24)	31	9	18
Vail (1)	3	1	3
<b>TOTAL</b>	<b>70</b>	<b>12</b>	<b>41</b>

<b>Suburban Sites</b>	<b># Samples</b>	<b># CS +</b>	<b># ELISA +</b>
Rincon Mountains, Rocking K Development	18	9	13
Tortolita Mountains, Saguaro Ranch Development	4	0	2
Tucson Mountains, Panther Peak Wash, SNPW	19	9	10
Tumamoc Hill	8	3	6
<b>TOTAL</b>	<b>49</b>	<b>21</b>	<b>31</b>

<b>High-Visitor Impact Sites</b>	<b># Samples</b>	<b># CS +</b>	<b># ELISA +</b>
Rincon Mountains, Mother's Day Fire, SNPE	25	10	12
Santa Catalina Mountains, Sabino Canyon Recreation Area	9	1	4
Tucson Mountains, Visitor Center, SNPW	4	1	1
<b>TOTAL</b>	<b>38</b>	<b>12</b>	<b>17</b>

<b>Remote Sites</b>	<b># Samples</b>	<b># CS +</b>	<b># ELISA +</b>
Ninetysix Hills	13	0	7
Black Mountain	17	8	10
Desert Peak	1	0	0
Rincon Mountains, Chiminea Creek	8	0	1
Sierrita Mountains, Stevens Canyon	9	4	3
Tortolita Mountains, Derrio Canyon	3	0	0
<b>TOTAL</b>	<b>51</b>	<b>12</b>	<b>21</b>

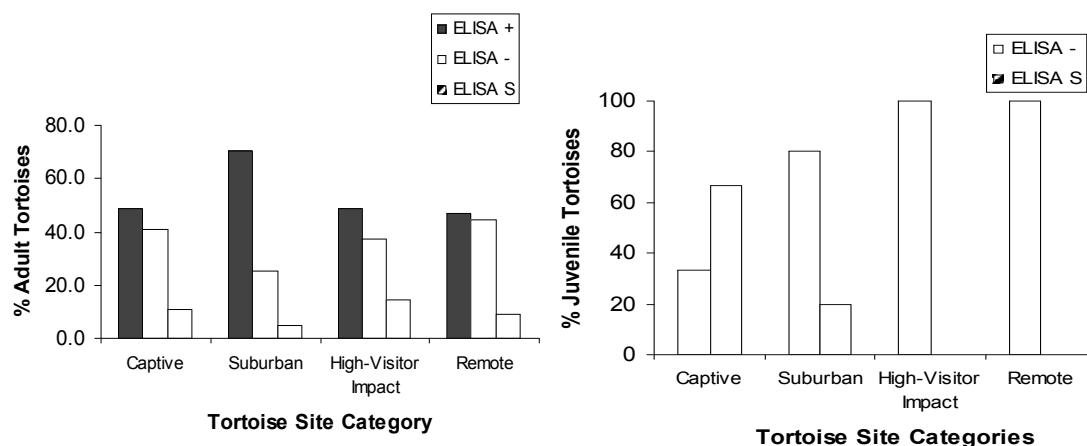
The overall number of adult and juvenile ELISA positive, negative, and suspect tortoises in each tortoise site category is summarized in Figure 3. Because suspect results are an intermediate value, and are inconclusive without retesting, we excluded all suspect animals from our statistical analyses.

ELISA results varied by tortoise site category, with the percentage of tortoises testing positive for *M. agassizii* antibodies higher closer to urban areas (Figure 4). Tortoises found in suburban sites had a significantly higher percentage of seropositivity than tortoises found in remote sites (Pearson chi-square = 4.5,  $p = 0.03$ ,  $df = 1$ ). A smaller percentage of captive tortoises were seropositive than suburban tortoises (Pearson chi-square = 3.898,  $p = 0.04$ ,  $df = 1$ ). ELISA results did not vary by sex (Pearson's chi-square = 1.03,  $p = 0.59$ ,  $df = 1$ ) but did vary by age class, with percentage of juvenile tortoises testing negative for *M. agassizii* significantly higher than adult tortoises (Pearson chi-square = 20.492,  $p = 0.0001$ ,  $df = 2$ ).

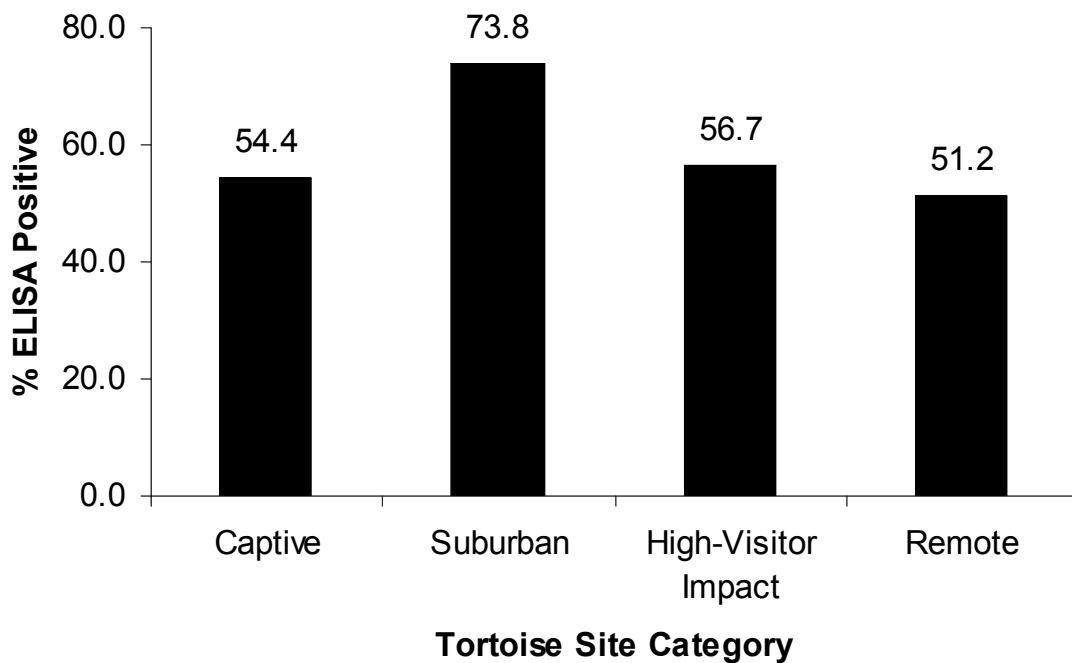
### **Clinical Signs Results.**

Of 57 tortoises that presented at least one clinical sign, 33 (80.5%) were ELISA positive and 8 (19.5%) ELISA negative. Of the 126 tortoises that did not present any clinical signs, 67 (53.2%) were ELISA positive and 59 (46.8%) ELISA negative. Clinical signs alone were not an effective test to predict positive ELISA results (Pearson chi-square = 9.61,  $p = 0.0019$ ,  $df = 1$ ). The lone tortoise that tested positive for antigens in the PCR test was also ELISA positive, but did not exhibit clinical signs.

Twelve (21%) tortoises (six captive and six free-ranging) presented five (of six possible) clinical signs for URTD that included wheezing, white or clear discharge, wet nares, sunken eyes, and eyelid swelling. Ten of these tortoises were ELISA positive, one ELISA suspect, and one ELISA negative. Three (5.3%) tortoises expressed three clinical signs (wet nares, sunken eyes, and eyelid swelling), of which two tested ELISA positive and one ELISA suspect. The remaining 42 tortoises expressed one or two ocular clinical signs (sunken eyes or eyelid swelling); 27 (64.3%) were ELISA positive, 12 (28.6%) ELISA negative, and 3 (7.1%) ELISA suspect.



**Figure 3.** Distributions of adult tortoises (left) and juvenile tortoises (right) with positive, negative and suspect ELISA results in Captive (n=63 adu, 7 juv), Suburban (n=44 adu, 5 juv), High-Visitor Impact (n=35 adu, 3 juv), and Remote (n=44 adu, 6 juv) site categories.



**Figure 4.** Percent ELISA positive results for adult Captive (n = 57), Suburban (n = 42), High-visitor impact area (n = 30), and Remote (n = 41) desert tortoises. Tortoises found in suburban sites were most likely to be seropositive. Tortoises with suspect results have been excluded.

#### **PCR Results.**

Only one of 205 nasal flush samples submitted for PCR tested positive for the *M. agassizii* DNA fingerprint. The remaining 204 results were negative, indicating that there was no correlation between the number of tortoises currently infected with *M. agassizii* (PCR) and tortoise site category.

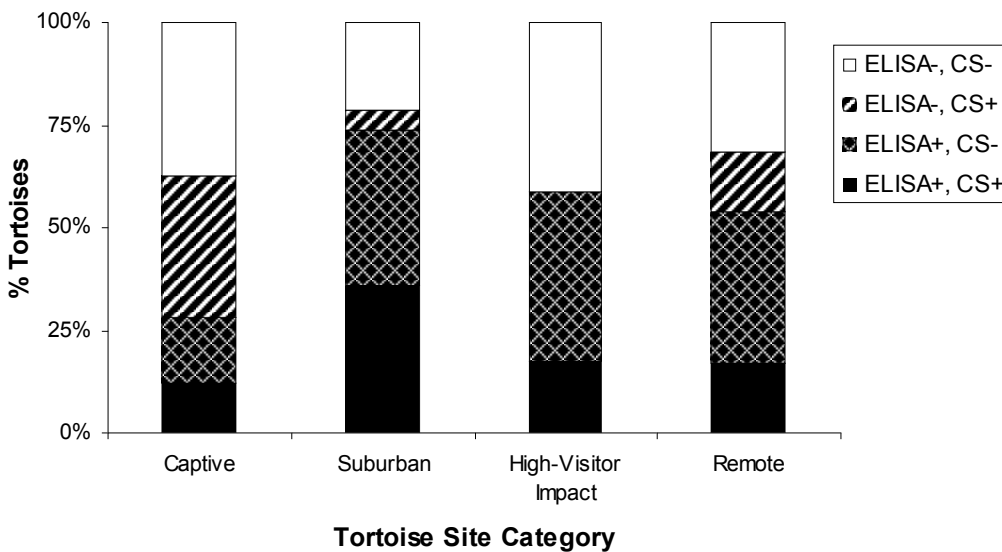
#### **Physical Exam Results.**

##### *Herpesvirus and Shell Disease Results.*

None of the 227 tortoises encountered expressed clinical signs for herpesvirus. Sixty (25.5%) tortoises showed some evidence of shell disease such as whitening of the scute seams, whitening between scales on the forelimbs, minor scute peeling, or pitting. Fifteen (6.4%) had one to four sand flies (*Lutzomyia tanyopsis*) on the head, limbs and / or carapace seams.

##### *Documentation of Harassment and Captivity.*

We documented 21 incidences of harassment by wild or domestic canids based on shell damage primarily on the marginal scutes above the limbs or the gular horns. Fifteen of these tortoises were missing one or both gular horns. One tortoise was missing digits on its hind limb, another was missing most of its forelimb. Four tortoises had evidence of punctures on the plastron and carapace consistent with mountain lion bite. Only one tortoise encountered had obvious evidence of previous captivity. This tortoise had the letter “D” painted on vertebral scutes 4-5.



**Figure 5.** Comparison of ELISA results with presence of clinical signs in tortoises from each category in the Greater Tucson Area. Results are expressed as the percentage of tortoises with positive or negative ELISA results in conjunction with the presence (CS+) or absence (CS-) of clinical signs. Tortoises with suspect results have been excluded.

### **Radio-telemetry.**

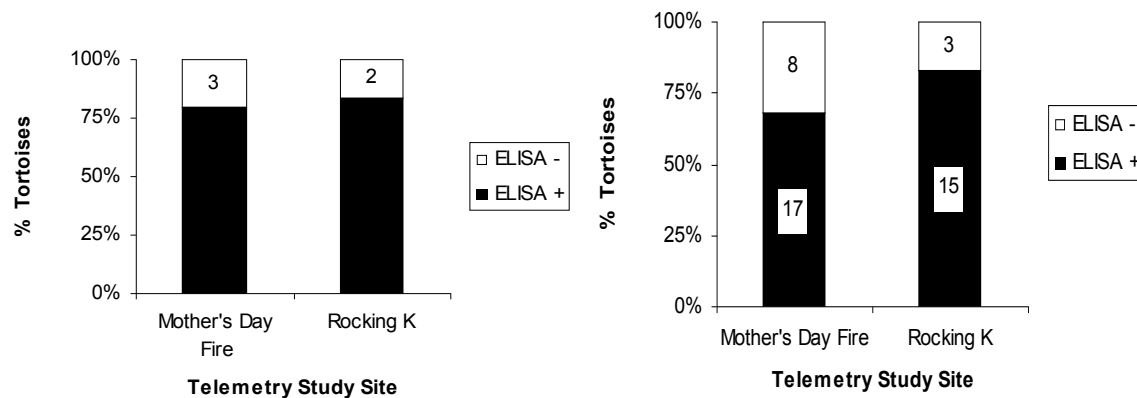
We affixed radio-transmitters to 11 adult tortoises and replaced radios on 19 tortoises at these sites, six of which presented at least one clinical sign of URTD.

At the Mother's Day Fire site (MDF), we radio-tracked 13 in 2002, 15 in 2003, and 11 in 2004. At the Rocking K (RK), we tracked 12 tortoises in 2002-2003, and 11 in 2004. Individual tortoises were located 12 to 89 (mean = 48) times in 2002–2004. One tortoise, adult female RK565, made a single, short term (6-day), long distance (11.24 km round trip) movement after laying eggs in July 2002 (Stitt 2004); location data for this movement were considered to be outliers and excluded from home range size analyses.

We collected blood samples from 25 tortoises at the MDF site and 18 at the RK. Of these, 15 were telemetered at the MDF and 12 at the RK. ELISA results for both telemetered and non-telemetered tortoises are summarized in Figure 6.

Of seven telemetered tortoises retested for ELISA in 2004, five remained positive, one changed from negative to positive, and one went from suspect to negative. Three of the tortoises that remained ELISA positive decreased in positive titer levels.

Two tortoises died during the study. The first, adult male MDF721, was submitted for necropsy at the Arizona Veterinary Diagnostic Lab on 14 Nov 2003, and found to have died of a bacterial bronchopneumonia, an infection in the lower respiratory tract, and not from *Mycoplasma* URTD (G. Bradley, DVM, personal communication 2003). The second tortoise, adult female RK486, was found decomposed in a burrow; the cause of death undetermined. The transmitter signals of 15 tortoises were lost, presumably due to premature transmitter failure.



**Figure 6.** Comparison of ELISA positive results for telemetered tortoises (left) and all tortoises sampled (right) at Mother's Day Fire (n = 25) and Rocking K (n = 18) study sites. Tortoises with suspect results are excluded.

#### *Home Range Size Estimates.*

ELISA positive tortoises had mean 100% MCP home range sizes of 9.8 ha (n = 17, 95% CI = 6.3 to 12.8) and ELISA negative tortoises had mean 100% MCP home ranges of 9.2 ha (n = 4, 95% CI = 2.7 to 15.6) (Appendix C). The 100% MCP home range size did not vary by ELISA result (linear regression,  $F_{1,19} = 0.02$ ,  $p = 0.89$ ) or sex (linear regression,  $F_{1,19} = 0.21$ ,  $p = 0.65$ ). The 100% MCP home range size estimates were smaller with fewer numbers of observations (linear regression,  $F_{1,19} = 7.64$ ,  $p = 0.01$ ).

ELISA positive tortoises had mean 95% kernel home range sizes of 9.1 ha (95% CI = 3.8 to 8.8) and ELISA negative tortoises had mean 95% kernel home ranges of 6.3 ha (95% CI = 3.9 to 14.3) (Appendix C). The 95% kernel home range size did not vary by ELISA result (linear regression,  $F_{1,19} = 0.91$ ,  $p = 0.35$ ), sex (linear regression,  $F_{1,19} = 0.05$ ,  $p = 0.83$ ), or number of observations (linear regression,  $F_{1,19} = 0.23$ ,  $p = 0.64$ ).

ELISA positive tortoises had mean 50% kernel home range sizes of 0.9 ha (95% CI = 0.4 to 1.0) and ELISA negative tortoises had mean 50% MCP home ranges of 1.1 ha (95% CI = 0.5 to 1.8) (Appendix C). The 50% kernel home range size did not vary by ELISA result (linear regression,  $F_{1,19} = 0.31$ ,  $p = 0.59$ ), sex (linear regression,  $F_{1,19} = 0.05$ ,  $p = 0.83$ ), or number of observations ((linear regression,  $F_{1,19} = 1.86$ ,  $p = 0.19$ ).

**Table 2.** Home range size estimates for ELISA positive and negative tortoises from 2002 to 2004 (Appendix C).

	ELISA + <sup>1</sup>		ELISA -	
	(n = 17)	95% CI	(n = 4)	95% CI
<b>100% MCP (ha)</b>	9.8 <sup>2</sup>	6.3 to 12.8	9.2	2.7 to 15.6
<b>95% Kernel (ha)</b>	9.1	3.3 to 8.3	6.3	4.2 to 14.1
<b>50% Kernel (ha)</b>	0.9	0.4 to 1.0	1.1	0.5 to 1.8

<sup>1</sup>4 outlying points for tortoise RK565 excluded

<sup>2</sup>95% MCP for tortoise RK565

**Thermal Ecology.**

Mean active season length for ELISA positive tortoises from 2002-2004 was 139.7 days ( $n = 33$ , 95% CI = 119.7 to 159.6). ELISA negative tortoises had a mean active season length of 146.6 days ( $n = 9$ , 95% CI = 108.5 to 184.9). The active season length during 2002-2004 did not vary by ELISA result (linear regression,  $F_{1,40} = 0.11$ ,  $p = 0.75$ ) or sex (linear regression,  $F_{1,40} = 0.23$ ,  $p = 0.63$ ). There was an interaction between active season length and year (linear regression,  $F_{1,40} = 3.11$ ,  $p = 0.056$ ), where tortoises were active longer in 2003 (Table 3). Four of the nine iButtons affixed to desert tortoises and seven of the eight placed in burrows (hibernacula) in December 2002 were recovered in 2003. Two of the iButtons not recovered from tortoises in 2003 were missing when the tortoises were first located after emerging from their over-winter burrows; we searched the area of each burrow, but were unable to recover them. We lost the transmitter signals of two tortoises equipped with iButtons, presumably due to premature transmitter failure. We were unable to relocate one iButton from an over-winter burrow in 2003.

Due to iButton program malfunction, no data was collected during the inactive season in 2003-2004.

All four of the tortoises with temperature data were ELISA positive and clinical sign negative. Two were males (MDF339 and MDF721) and two were females (MDF289 and MDF410). Two of the tortoises (MDF339 and MDF410) shared an over-winter burrow, and therefore were monitored with a single burrow iButton.

MDF721's iButton recorded higher temperatures than the burrow iButton daily from 7 December 2002-7 February 2003, again on 9 February-11 February 2003, and daily from 14 February-10 March 2003 when the iButton stopped recording data. This indicates that this tortoise was basking 90 out of 93 days during the inactive season; it was observed basking on 11 January and 1 February 2003. This tortoise was not expressing clinical signs before entering the winter (inactive) season, but began to appear emaciated in September and was found dead on 13 November 2003. A necropsy identified bacterial bronchopneumonia as cause of death.

Of the two tortoises sharing a burrow, the iButton on MDF339 recorded higher temperatures than the burrow iButton 18 December 2002-03 January 2003, then again on 6 and 26 February 2003, and 3 March 03. This indicates that MDF339 was basking 24 out of 90 days during the inactive season. MDF410's iButton recorded higher temperatures than the burrow iButton on 19, 24-25, and 27 December 2002, and again on 19 March 2003. This indicates that this tortoise was basking 5 out of 90 days during the inactive season.

The iButton on MDF298 recorded temperatures within 1-4°F of the burrow iButton 14 December 2002-11 February 2003, higher than the burrow on 12 February 2003, then 1-4°F lower than the burrow 13 February-17 March 2003. This indicates that MDF298 was basking one out of 90 days during the inactive season.

**Table 3.** Mean number of days in each active season for tortoises from 2002 – 2004.

Year	2002		2003		2004	
Length of Active Season (days)	116		167.3		140.2	
95% Confidence Interval	87.2 to	144.8	138.5 to	196.6	111.4 to	169
Sample Size	14		14		14	

## DISCUSSION

### **Tortoise Sample Groups.**

Of the 64 blood samples collected from adult captive desert tortoises in this study, 48.4% tested ELISA positive. This value is lower than results from two sites in California where 60–61.8% of captive tortoises tested positive for the *M. agassizii* antibodies (Berry et al. 2003). Captive tortoises in our study had the second lowest percentage of ELISA positive results, almost equal to remote tortoises. Although this does not indicate whether or not captive tortoises are the original disease source for the wild population, our data does suggest that captive tortoises are not currently an important reservoir of *M. agassizii* for the wild population around Tucson. A very high percentage of free-ranging suburban tortoises have already been infected. Explanations for the high percentage of seropositivity we detected in suburban tortoises are speculative. The high disease incidence may be related to anthropogenic or environmental stress caused by urbanization.

Of the 124 blood samples collected from free-ranging adult desert tortoises in this study, 55.7% were seropositive for exposure to *M. agassizii*. This value is higher than results from a study conducted on free-ranging desert tortoises in Las Vegas Valley (Nevada, USA), where 144 tortoises were sampled and 50% were seropositive (Schumacher et al. 1997). This value is also much higher than found in two health studies conducted in Arizona in 1990 and 2002. In 1990, 3% of tortoises sampled at Littleship Wash and Harcuvar Mountains were seropositive (Dickinson et al. 2002). In 2002, 8.7% of tortoises sampled from various monitoring plots (Florence Military Reservation, Ragged Top Mountain, SNPE, Sugarloaf Mountain, Bonanza Wash, Buck Mountain, East Bajada, Harcuvar mountains, San Pedro Wash, and West Silverbells) were seropositive (Riedle and Averill-Murray, 2003); only tortoises adjacent to urban areas (Ragged Top Mountain, SNPE) were seropositive, all tortoises from isolated areas tested ELISA negative.

### **ELISA.**

When comparing percentages of ELISA positive tortoises from this study, there was a positive relationship between proximity of tortoises to suburban sites and exposure to *M. agassizii* as measured by ELISA. Free-ranging tortoises in suburban areas had the highest percentage of *M. agassizii*, remote areas had the lowest, and high-visitor impact areas were intermediate, which indicates that there is an urban gradient associated with free-ranging desert tortoises and URTD.

All juveniles included in this study were either ELISA negative or suspect. URTD is fatal to smaller tortoises, so it is possible that the juvenile tortoises we encountered had not been exposed to *M. agassizii* (M. Brown, personal communication 2004). These suspect, or inconclusive, results could be due to smaller blood volume collected from smaller tortoises.

Though five of the seven telemetered tortoises retested for ELISA in 2004 remained positive, three had decreases in positive titer levels. When these three tortoises were first tested, their titer levels were high positives (256); at the time of retest they were each moderate positives (128). It is not uncommon for titer levels to fluctuate over time, increasing when antibodies levels are high (indicating a recent infection) and decreasing when antibodies are lower (indicating a time lapse from previous infection) (M. Brown personal communication, 2004). The other two tortoises' titer levels changed, one from negative to positive, the other from suspect to negative.

### **Clinical Signs.**

Of the 208 tortoises sampled in this study, only 57 (27.4%) expressed clinical signs of a current URTD infection yet 48% were ELISA positive. Of the 151 tortoises not expressing clinical signs, 53.2% tested ELISA positive. Though other URTD studies have found a positive relationship between clinical signs and seropositivity (Schumacher et al. 1997), our results do not indicate that presence or absence of clinical signs is a reliable indicator of the presence or absence of *M. agassizii* antibodies.

Clinical signs may not be a reliable tool to diagnose URTD because they appear within one or two weeks post-exposure to *M. agassizii*, but it takes six to eight weeks for an exposed tortoise to develop antibodies detectable by an ELISA (McLaughlin 2000; Diemer Berish 2000). However, given the lifespan of desert tortoises, it seems unlikely that we happened to draw blood samples from tortoises during this 4–7 week window. Clinical signs fluctuate over time in diseased tortoises, which also makes them unreliable. Some clinical signs, including nasal discharge and conjunctivitis, can also be characteristic other non-URTD health conditions such as dehydration, poor nutrition, heat stress, infection of herpesvirus or another bacteria (Chlamydia or Pastuerella) (Schumacher et al 1997; McLaughlin 2000). Wet nares can be caused by recent ingestion of food or water, (McLaughlin 2000).

### **PCR.**

Only 15 of the 205 tortoises sampled were expressing clinical signs that included nasal discharge and wet nares at the time of nasal flush sampling, and none were PCR positive. PCR results are highly dependent on the quality of the sample. In desert tortoises, the mucosal surfaces of ventrolateral recesses in the nasal passage, the preferential site of bacterial growth, is not easily sampled by nasal flush, especially under field conditions. PCR-negative results could indicate that *Mycoplasma* organisms were not present at time of sampling, or were present but in low numbers and the sampling technique failed to collect them.

### **Physical Exam.**

#### *Documented Harassment or Captivity.*

We located 21 tortoises with shell or limb damage that could be attributed to wild or domestic canids. Observations of severe damage (pieces chewed off of gular or marginals) have been attributed to domestic dogs (A. Demnon, unpublished data 2004; Edwards 2004). Of these 21, 57% were found at suburban sites. At two suburban study areas, Panther Peak Wash, and Tumamoc Hill, where shell damage was most severe, we encountered packs of 3-5 presumably feral dogs. At Panther Peak Wash, we heard dogs barking, and saw them in an area where a tortoise was found bleeding from the carapace and plastron.

### **Radio-telemetry.**

#### *Home Range Size Estimates.*

The estimated 100% MCP home range sizes are similar to others in the Sonoran Desert (Bailey 1992, Trachy and Dickinson 1993, Averill-Murray and Klug 2000). Though our 100 % MCP home range estimates are comparable to other studies, 100% MCP is particularly influenced by small sample size. There was an interaction between the number of observations and 100% MCP, where we found that home range sizes were smaller with fewer observations. Three of the tortoise home range sizes included in our estimates were calculated based on 12 to

19 observations, and were present in our study for less than one year. We included these three tortoises in our analyses because we feel we adequately captured their active season with the number of observations recorded.

95% and 50% kernel home range sizes can be estimated with as few as 10 observations, though more than 50 observations are considered optimal (Aebischer et al. 1993, Garton et al. 2001, Kernohan et al. 2001). There were no interactions between number of observations and either 95% or 50% kernel home range estimates.

ELISA positive tortoises did not have a significantly different 100% MCP, 95% kernel, or 50% kernel home range size than ELISA negative tortoises. These results could be influenced by the smaller number of ELISA negative (4) tortoises in our study, or ELISA positive tortoises with small home range sizes (RK404 spent most of the year inactive in his burrow and made no large movements).

### **Thermal Ecology.**

There was no difference in the mean active season length for ELISA positive and negative tortoises regardless of sex from 2002-2004. We tracked tortoises every 7-10 days in the active season (March-October), so length of our active season has an error of 14-20 days. Our mean active season length over the duration of the study was 4.5 months. Though the length of the active season varied by year, with the shortest active season in 2002 (3.8 months) and the longest in 2003 (5.5 months), all three years are comparable to other desert tortoise studies which found active season lengths of 3.5 – 5.5 months (Burge 1977, Vaughn 1984, Bailey et al. 1995, Martin 1995, Stitt 2004).

Though the length of the active season for our tortoises was comparable to other tortoise studies, the tortoises with iButtons were not found in the open until June–September. Because we did not want to disturb tortoises in burrows, we were unable to download temperature data every two months, as originally planned, to determine temperature selection in both the active and inactive season. Instead, we removed the iButtons when each tortoise was in the open or under vegetation during the active season, and affixed iButtons to available tortoises just before their over-winter hibernation.

We were only able to recover four tortoise–burrow pairs of iButtons from 2002-03 to compare over-winter temperature selection of tortoises. All four of these tortoises tested ELISA positive and clinical sign negative, so we were unable to compare temperature selection of tortoises with and without UR TD.

We found that winter activity varies greatly by individuals, which is consistent with the results of other studies in which most tortoises remain in their hibernacula throughout the winter (inactive) season, while others remain active on warmer winter days (Burge 1977, Vaughn 1984, Bailey et al. 1995, Martin 1995). The two females we monitored were rarely active, one apparently basked one day in February 2003, and the other basked four days in December 2003 and one in March 2003. Of the two males we monitored, one male recorded temperatures that indicated he basked daily for 21 days in December-January, then again for three days in February, and one day in March 2003, while the other apparently basked daily with the exception of three days in February 2003. While the latter tortoise (MDF721) was not exhibiting any clinical signs of UR TD, he died in November 2003 of bacterial bronchopneumonia. Other researchers have observed clinically ill tortoises remaining active throughout the winter, coming out to bask, forage, and drink (S. Bailey personal observation, J. Jarchow personal communication, E. Zylstra personal observation, J. Capps personal observation).

Our temperature data would have been improved by increasing the number of iButtons used in both the burrow and surrounding environment, and implementing a deflector for iButtons in burrows (i.e., aluminum foil). Additional iButtons in various locations in burrows may help better explain small temperature differences between tortoises and burrows, and additional iButtons in the environment may help identify where tortoises go when they emerge over-winter. Adding deflectors to burrow iButtons would decrease variation in temperature that was due to direct sunlight. One iButton placed in a west facing burrow, shared by MDF410 (female) and MDF339 (male), recorded daily temperature increases of up to 22.9°F over both tortoise temperatures at 4:32 PM each day in December 2002–January 2003; this time and temperature combination corresponds with sunlight from the setting sun entering the burrow.

We attempted to program all iButtons to disable the rollover function which would allow data to be written over once the memory is full; however, all iButtons programmed in December 2003 did rollover and wrote over the winter temperature recorded data. Once the data is written over, there is no way to recover it (K. Edmundson, Dallas Semiconductor, personal communication 2004).

## MANAGEMENT AND RESEARCH IMPLICATIONS

URTD has been identified as a major contributing factor in the decline of the desert tortoise in the Mojave Desert (Jacobson et al. 1991). Though other studies in the Sonoran Desert have found little to no presence of URTD, we found *M. agassizii* in 12 of 13 sites sampled, showing that URTD is prevalent in and around Tucson, AZ. Furthermore, infection rates are highest near urban areas. Because we found *M. agassizii* in most of our study sites, we recommend continued research on long-term monitoring plots to track trends related to disease incidence, mortality, and status of populations. We also recommend research on anthropogenic stressors that may be the cause of the higher percentage of *M. agassizii* in suburban areas. Though *M. agassizii*-infected captive tortoises are not likely to currently pose a major threat to wild tortoise populations around Tucson, we recommend an increase in education outreach about the potential danger of releasing captive tortoises and more stringent guidelines for adopting desert tortoises.

### **Monitoring desert tortoise populations for disease.**

A combination of complete physical exam and laboratory tests will best identify clinically ill tortoises. ELISA provides an effective, rapid, and inexpensive way to detect specific antibodies associated with exposure to *M. agassizii*. ELISA results provide information about the overall distribution and prevalence of *M. agassizii* antibodies, and can be used to assess and compare relative health status of desert tortoises in a population or region. Because URTD can be clinically silent, changes in percentage of ELISA positive tortoises could provide an early warning for potential disease outbreaks in populations. Knowledge of the distribution of *M. agassizii* in populations will allow better management decisions concerning populations which are potentially at risk to acquire or to spread URTD. When possible, additional plasma samples should be submitted for other ELISA tests to detect the presence of antibodies indicating exposure to herpesvirus and *Mycoplasma testudinidae*.

### **Research on anthropogenic stressors.**

URTD may be a secondary factor in tortoise declines in the Mojave Desert; other factors need to be considered and monitored as well (Lovich and Bainbridge 1999). Stress caused by the

many disturbances associated with urban encroachment may make the tortoises especially vulnerable to disease that is already present in the wild populations. The disease gradient we observed for desert tortoises in the Tucson area is consistent with this hypothesis. At this time, published data are available on effects of environmental changes such as seasonal changes, drought, and high precipitation levels on desert tortoises (Lovich and Bainbridge 1999, Edwards et al. 2004, Stitt 2004). However, little data is available regarding the effects of anthropogenic disturbances, such as human expansion into previously uninhabited areas, habitat fragmentation and degradation due to urban expansion, and increased contact with humans and domestic pets. Further research is needed to examine the relationship between anthropogenic stressors and URTD.

### **Captive desert tortoises.**

Although URTD is more common in free-ranging suburban than captive tortoise populations in the Tucson area, captive tortoises were implicated in the spread of URTD in Mojave desert tortoises and may have initially played a role as a disease vector in the Sonoran Desert as well. The highest prevalence of URTD in California was found in two study sites where previous releases of captive desert tortoises have occurred (Jacobson et al. 1995). The release of captive desert tortoises may contribute to the spread of new infectious diseases to wild tortoises. Though laws already exist in Arizona prohibiting the release of captive tortoises, the information needs to be disseminated to each desert tortoise custodian at the time of adoption, and again every few years to promote compliance.

Tortoise adoption programs provide homes for displaced desert tortoises. Once tortoises are adopted, there is little to no follow-up done by the agencies running the programs. These agencies may need to implement education outreach protocols that involve checking on captive desert tortoise care by either conducting yard checks or contacting custodians. During communications with custodians, agencies should stress the importance of not breeding or releasing desert tortoises, and housing all turtle and tortoise species separately to prevent spread of diseases across different species.

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### LITERATURE CITED

- Aebischer, N. J., P. A. Robertson, and R. E. Kenward. 1993. Compositional analysis of habitat use from animal radio-tracking data. *Ecology* 74:1313-1325.
- Arizona Game and Fish Department. 1996. Wildlife of special concern in Arizona. Nongame and Endangered Wildlife Program, Arizona Game and Fish Department, Phoenix, Arizona. 40 pp.
- Arizona Interagency Desert Tortoise Team. 1996a. Management plan for the Sonoran Desert population of the desert tortoise in Arizona. R. C. Averill-Murray, editor. Arizona Game and Fish Department and US Fish and Wildlife Service, Phoenix, Arizona. December: 55 pp.
- \_\_\_\_\_. 1996b. Protocols for handling free-ranging Sonoran Desert tortoises. July revision: 17 pp.
- \_\_\_\_\_. 2000. Status of the Sonoran population of the desert tortoise in Arizona: an update. R. C. Averill-Murray, editor. Arizona Interagency Desert Tortoise Team and Arizona Game and Fish Department, Phoenix, Arizona. 48 pp.
- Averill-Murray, R. C., and C. M. Klug. 2000. Monitoring and ecology of Sonoran desert tortoises in Arizona. Nongame and Endangered Wildlife Program Technical Report 161. Arizona Game and Fish Department, Phoenix, Arizona, USA.
- \_\_\_\_\_, B. E. Martin, S. J. Bailey, and E. B. Wirt. 2002. Activity and behavior of the sonoran desert tortoise in Arizona. Pages 312-333 *in* the Sonoran desert tortoise: natural history, biology and conservation. T. R. Van Devander, ed. University of Arizona Press, Tucson, AZ, USA.
- Bailey, S. J., C. R. Schwalbe, and C. H. Lowe. 1995. Hibernacula use by a population of desert tortoises (*Gopherus agassizii*) in the Sonoran Desert. *Journal of Herpetology* 29:361-369.
- Barrett, S. L. 1990a. Home range and habitat of the desert tortoise (*Xerobates agassizii*) in the Picacho Mountains of Arizona. *Herpetologica* 46(2): 202-206.
- \_\_\_\_\_, J. D. Humphrey, and S. D. Harper. 1990b. Desert tortoise reintroduction and mitigation assessment study: final report. Report to Bureau of Reclamation, Arizona Projects Office, Central Arizona Project, Phoenix, Arizona. 15 pp.
- Berry, K. H. 1990. The status of the desert tortoise (*Gopherus agassizii*) in California in 1989. Draft report to the U. S. Fish and Wildlife Service, Portland, Oregon, USA.
- \_\_\_\_\_. 1997. Demographic consequences of disease in two desert tortoise populations in California. Pages 91-99 *in* Jim Van Abbema, editor. Proceedings: conservation, restoration, and management of tortoises and turtles - an international conference. New York Turtle and Tortoise Society, New York, USA.

- \_\_\_\_\_, and T. Goodlett. 2000. Desert tortoise workshop and training materials: instructions for ordering equipment and culture media; summary of how to draw blood and conduct nasal lavages; and information on how to contract for laboratory analysis and ship laboratory samples. Prepared for Desert Tortoise Workshop on Health Evaluations, October 21-22, 2000. June 2001 revision: 5 pp.
- \_\_\_\_\_, M. B. Brown, L. Wendland, F. Origgi, A. Johnson. 2003. Health assessments of captive and wild desert tortoises at 26 sites in the Mojave and Colorado Deserts, California, in 2002. Abstract in Proceedings of the 2002 & 2003 Desert Tortoise Council Symposia.
- Bjurlin, C. D., and J. A. Bissonette. 2001. The impact of predator communities on early life history stage survival of the desert tortoise at the Marine Corps Air Ground Combat Center, Twentynine Palms, California. United States Department of the Navy Contract N68711-97-LT-70023. UCFWRU Pub. # 00-1:1-81.
- Boarman, W. I., T. Goodlett, G. Goodlett, and P. Hamilton. 1998. Review of radio transmitter attachment techniques for turtle research and recommendations for improvement. *Herpetological Review* 29(1): 26-33.
- Brown, D. R., B. C. Crenshaw, G. S. McLaughlin, I. M. Schumacher, C. E. McKenna, P. A. Klein, E. R. Jacobson, and M. B. Brown. 1995. Taxonomic analysis of the tortoise mycoplasmas *Mycoplasma agassizii* and *Mycoplasma testudinis* by 16s rRNA gene sequence comparison. *International Journal of Systematic Bacteriology* 45:348-350.
- Brown, M. B., I. M. Schumacher, P. A. Klein, K. Harris, T. Correll, and E. R. Jacobson. 1994. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. *Infection and Immunity* 62:4580-4586.
- Christopher, M. M., K. Nagy, I. Wallis, and K. H. Berry. 1997. Laboratory health profiles of desert tortoises in the Mojave Desert: a model for health status evaluation of chelonian populations. Pages 76-82 in J. Van Abbema editor. Proceedings: Conservation, restoration, and management of tortoises and turtles - an international conference. Wildlife Conservation Society Turtles Recovery Program and the New York Turtle and Tortoise Society, Purchase, New York, USA.
- Dickinson, V. M., J. L. Jarchow, M. H. Trueblood, and J. C. deVos. 2002. Are free-ranging Sonoran Desert Tortoises healthy? Pages 242-264 in the Sonoran desert tortoise: natural history, biology and conservation. T. R. Van Devander, ed. University of Arizona Press, Tucson, AZ, USA.
- Diemer Berish, J. E., L. D. Wendland, and C. A. Gates. 2000. Distribution and prevalence of upper respiratory tract disease in gopher tortoises in Florida. *Journal of Herpetology* 34:5-12.
- Edwards, T., C. R. Schwalbe, D. E. Swann, and C. S. Goldberg. 2004. Implications of anthropogenic landscape change on inter-population movements of the desert tortoise. *Conservation Genetics* 5:485-499.
- Esque, T. C., A. Burquez M., C. R. Schwalbe, T. R. Van Devander, P. J. Anning, and M. J. Nijuis. 2002. Fire ecology of the Sonoran desert tortoise. Pages 312-333 in the Sonoran desert tortoise: natural history, biology and conservation. T. R. Van Devander, ed. University of Arizona Press, Tucson, AZ, USA.
- \_\_\_\_\_, C. R. Schwalbe, L. A. DeFalco, R. B. Duncan, and T. J. Hughes. 2003. Effects of desert wildfires on desert tortoise (*Gopherus agassizii*) and other small vertebrates. *Southwestern Naturalist* 48:103-111.

- Fowler, M. E. 1976. Respiratory disease in desert tortoises. Pages 79-99 in N. J. Engberg, S. Allan, and R. L. Young editors. Proceedings of the Symposium of the Desert Tortoise Council, Long Beach, California, USA.
- Garton, E. O., M. J. Wisdom, F. A. Leban, and B. K. Johnson. 2001. Pages 15-42 in J. J. Millspaugh and J. M. Marzluff, editors. Radio tracking and animal populations. Academic Press, San Diego, CA, USA.
- Hooge, P. N., B. Eichenlaub, and E. Solomon. 1999. The animal movement program. USGS Alaska Biological Science Center, Anchorage, Alaska, USA.
- Jacobson, E. R., J. M. Gaskin, M. B. Brown, R. K. Harris, C. H. Gardiner, J. L. LaPointe, H. P. Adams, and C. Reggiardo. 1991. Chronic upper respiratory tract disease of freeranging desert tortoises, *Xerobates agassizii*. Journal of Wildlife Diseases 27:296-316.
- \_\_\_\_\_, J. Schumacher, and M. Green. 1992. Field and clinical techniques for sampling and handling blood for hematologic and selected biochemical determinations in the desert tortoise, *Xerobates agassizii*. Copeia 1:237-241.
- \_\_\_\_\_. 1993. Implications of infectious diseases for captive propagation and introduction programs of threatened/endangered species. Journal of Zoo and Wildlife Medicine 24:245-255.
- \_\_\_\_\_, M. B. Brown, I. M. Schumacher, B. R. Collins, R. K. Harris, and P. A. Klein. 1995. Mycoplasmosis and the desert tortoise (*Gopherus agassizii*) in Las Vegas Valley, Nevada. Chelonian Conservation and Biology 1:279-284.
- Kernohan, B. J., R. A. Gitzen, and J. J. Millspaugh. 2001. Pages 125-166 in J. J. Millspaugh and J. M. Marzluff, editors. Radio tracking and animal populations. Academic Press, San Diego, CA, USA.
- Knowles, C. 1989. A survey for diseased desert tortoises in and near the Desert Tortoise Natural Area, Spring 1989. Report prepared for the Bureau of Land Management, Riverside, California, USA. Contract No. CA 950-(T9-23), 26 pp.
- Lederle, P.E., K.R. Rautenstrauch, D.L. Rakestraw, K.K. Zander, and J.L. Boone. 1997. Upper respiratory tract disease and mycoplasmosis in desert tortoises from Nevada. Journal of Wildlife Diseases 33:759-765.
- Lovich, J. E., D. Bainbridge. 1999. Anthropogenic degradation of the Southern California desert ecosystem and prospects for natural recovery and restoration. Environmental Management 24:309-326.
- Martin, B. E. 1995. Ecology of the desert tortoise (*Gopherus agassizii*) in a desert-grassland community in southern Arizona. M.S. Thesis, University of Arizona, Tucson.
- McLaughlin, G. S., E. R. Jacobson, D. R. Brown, C. E. McKenna, I. M. Schumacher, H. P. Adams, M. B. Brown, and P. A. Klein. 2000. Pathology of upper respiratory tract disease of gopher tortoise in Florida. Journal of Wildlife Diseases 36:272-283.
- Murray, R. C., and C. R. Schwalbe. 1997. Second survey of the Four Peaks desert tortoise monitoring plot: testing abundance estimation population. Final report for Arizona Game and Fish Department Heritage IIPAM Project I95041, Phoenix, Arizona. 70 pp.
- Riedle, J. D., and R. C. Averill-Murray. 2003. Disease incidence on Sonoran Desert long term monitoring plots. Pages 141-146 in Proceedings of the 2002 & 2003 Desert Tortoise Council Symposia.
- Roskopf, W. J., E. Howard, A. P. Gendron, E. Walder, and J. O. Britt. 1981. Mortality studies on *Gopherus agassizii* and *Gopherus berlandieri* tortoises. Pages 108-112 in K. A. Hashagan editor. Proceedings of the Desert Tortoise Council Symposium.

- Schumacher, I. M., M. B. Brown, E. R. Jacobson, B. R. Collins, and P. A. Klein. 1993. Detection of antibodies to a pathogenic mycoplasma in desert tortoise (*Gopherus agassizii*) with upper respiratory tract disease. *Journal of Clinical Microbiology* 31:1454-1460.
- \_\_\_\_\_, D. B. Hardenbrook, M. B. Brown, E. R. Jacobson, and P. A. Klein. 1997. Relationship between clinical signs of upper respiratory tract disease and antibodies to *Mycoplasma agassizii* in desert tortoises from Nevada. *Journal of Wildlife Diseases* 33:261-266.
- Stitt, E. 2004. Demography, reproduction, and movements of desert tortoises (*Gopherus agassizii*) in the Rincon Mountains, Arizona. M.S. Thesis, University of Arizona, Tucson.
- Swann, D. E., C. R. Schwalbe, T. A. Volz, and D. B. Prival. 2001. Effect of urban development on desert tortoises: summary of 2000 field season. Unpublished report to T & E Incorporated, Cortaro, Arizona, USA. 14 pp.
- \_\_\_\_\_, R. C. Averill-Murray, C. R. Schwalbe. 2002. Distance sampling for Sonoran Desert Tortoises. *Journal of Wildlife Management* 66:969-975.
- U. S. Fish and Wildlife Service. 1989. Endangered and threatened wildlife and plants; determination of threatened status of the Mojave population of the desert tortoise desert tortoise. *Federal Register* 54(149)32326.
- \_\_\_\_\_. 1990. Endangered and threatened wildlife and plants; determination of threatened status of the Mojave population of the desert tortoise. *Federal Register* 55:12178-12191.
- \_\_\_\_\_. 1994. Desert tortoise (Mojave population) recovery plan. U.S. Fish and Wildlife Service, Portland, Oregon, USA. 73 pp. plus appendices.
- Vaughn, M. 1984. Home range and habitat use of the desert tortoise (*Gopherus agassizii*) in the Picacho Mountains, Pinal County, Arizona. M.S. Thesis, Arizona State University, Tempe.
- White, G. C., and R. A. Garrott. 1990. *Analysis of Wildlife Radio-tracking Data*. Academic Press, Inc., Toronto, Canada.
- Worton, B. J. 1989. Kernel methods for estimating the utilization distribution in home range studies. *Ecology* 70:164-168.
- Zar, J. H. 1996. *Biostatistical Analysis*, 3<sup>rd</sup> edition. Prentice Hall, Upper Saddle River, New Jersey, USA.

**APPENDIX A.** Letter to desert tortoise custodians requesting participation in research.

23 May 2003

Dear Desert Tortoise Custodian:

My name is Cristina Jones and I am a master's student at the University of Arizona studying Sonoran Desert tortoises. Your name was selected at random from a list provided by the Arizona Sonora Desert Museum Tortoise Adoption Program. I am writing to invite you to participate in my research and contribute to the conservation of this marvelous species. My investigation, funded by the Arizona Department of Game and Fish Heritage Program, and in cooperation with the Arizona-Sonora Desert Museum, National Park Service, University of Arizona, and James L. Jarchow, DVM, seeks to document the prevalence of upper respiratory tract disease (URTD) in captive and free-ranging tortoise populations in and around Tucson. Caused by the pathogen *Mycoplasma agassizii*, URTD is found in several turtle and tortoise species, and has contributed to desert tortoise declines in some areas. This study will provide important baseline information on the distribution of URTD in the Sonoran population of desert tortoises, particularly along the urban/wildland interface.

I am seeking permission from you to collect a small blood and nasal flush sample from your desert tortoise to test for URTD. If you agree to participate in this research/conservation effort, please either call (work: 621-5508, home: 320-1622), or send e-mail (cajones@u.arizona.edu), with a message that includes your name and telephone number, and I will phone you to schedule an appointment. For my samples, I draw 0.5 – 1.0 ml of blood from the brachial vein of either front leg and flush sterile saline into and out of the nostrils. The blood and nasal flush samples are shipped to the University of Florida for testing. Minimizing trauma to your tortoise and the wild tortoises in my study is my highest priority. I have been trained in the sampling techniques by Tucson tortoise specialist James L. Jarchow, DVM, and have been volunteering in the Herpetology Department of the Desert Museum for five years. If you have already scheduled a post-hibernation check-up for your desert tortoise with Dr. Jarchow, he is prepared to take the blood and nasal flush sample for me at the time of your appointment.

I have enclosed my business card; please do not hesitate to contact me with any questions via phone or e-mail. I look forward to meeting you and your tortoise.

Sincerely,

Cristina A. Jones  
Graduate Student Assistant  
Wildlife and Fisheries Science  
Enclosures (1)



**APPENDIX B.** Desert tortoise location and capture data.

Table B1. Location and capture data for tortoises sampled in the Greater Tucson Area, with clinical sign, ELISA, and PCR results.

Date	Tort ID	Location	Category	Sex	MCL (mm)	Weight (g)	Clinical Signs	ELISA Results	PCR Results
31-Aug-02	MDF339	MDF, SNPE	H-V Impact	♂	261	2984	No	+	-
31-Aug-02	MDF711	MDF, SNPE	H-V Impact	♀	263	2200	Yes	S	-
31-Aug-02	MDF712	MDF, SNPE	H-V Impact	♂	243	2600	No	-	-
21-Sep-02	MDF147	MDF, SNPE	H-V Impact	♂	273	4000	Yes	S	-
21-Sep-02	MDF410	MDF, SNPE	H-V Impact	♀	242	2600	Yes	+	-
21-Sep-02	MDF422	MDF, SNPE	H-V Impact	♂	265	3414	Yes	+	-
21-Sep-02	MDF721	MDF, SNPE	H-V Impact	♂			Yes	+	-
28-Sep-02	MDF126	MDF, SNPE	H-V Impact	♀	229	2120	No	+	-
28-Sep-02	MDF204	MDF, SNPE	H-V Impact	♀	208	1398	No	+	-
28-Sep-02	MDF207	MDF, SNPE	H-V Impact		167	722	No	-	-
28-Sep-02	MDF208	MDF, SNPE	H-V Impact	♂	252	2500	Yes	+	-
28-Sep-02	MDF209	MDF, SNPE	H-V Impact	♂	210	1460	No	+	-
28-Sep-02	MDF293	MDF, SNPE	H-V Impact	♂	227	1890	No	S	-
28-Sep-02	MDF298	MDF, SNPE	H-V Impact	♀	236	2160	No	+	-
16-Aug-03	MDF107	MDF, SNPE	H-V Impact		154	800	No	-	-
16-Aug-03	MDF214	MDF, SNPE	H-V Impact	♀	245	2500	No	-	-
16-Aug-03	MDF219	MDF, SNPE	H-V Impact		151	750	Yes	-	-
16-Aug-03	MDF233	MDF, SNPE	H-V Impact	♂	245	2400	Yes	S	-
16-Aug-03	MDF271	MDF, SNPE	H-V Impact	♂			No	-	-
16-Aug-03	MDF390	MDF, SNPE	H-V Impact	♂	259	3100	No	+	-
16-Aug-03	MDF508	MDF, SNPE	H-V Impact	♀	231	2100	Yes	+	-
11-Aug-04	MDF217	MDF, SNPE	H-V Impact	♀	215	1900	No	+	-
11-Aug-04	MDF300	MDF, SNPE	H-V Impact	♂	204	1850	No	-	-
11-Aug-04	MDF722	MDF, SNPE	H-V Impact	♀	234	2300	No	S	-
12-Aug-04	MDF221	MDF, SNPE	H-V Impact	♂	243	2500	No	-	-
20-Sep-03	SC03	Sabino Canyon	H-V Impact	♂	245	2650	No	+	-
20-Sep-03	SC04	Sabino Canyon	H-V Impact	♂	239	2300	Yes	+	-
5-Oct-03	SC05	Sabino Canyon	H-V Impact	♂	228	2000	No	+	-
5-Oct-03	SC16	Sabino Canyon	H-V Impact	♂	264	3500	No	+	-
19-Oct-03	SC22	Sabino Canyon	H-V Impact	♀	216	1850	No	-	-
19-Oct-03	SC23	Sabino Canyon	H-V Impact	♀	182	1000	No	-	-
17-Jul-04	SC02	Sabino Canyon	H-V Impact	♂	221	2100	Yes	-	-
17-Jul-04	SC42	Sabino Canyon	H-V Impact	♀	212	1600	No	-	-
17-Jul-04	SC43	Sabino Canyon	H-V Impact	♀	185	1100	No	-	-
18-Jul-04	SNPW300	Vis. Ctr, SNPW	H-V Impact	♀	224	1750	No	-	-
18-Jul-04	SNPW310	Vis. Ctr, SNPW	H-V Impact	♂	292	3750	Yes	+	-
18-Jul-04	SNPW311	Vis. Ctr, SNPW	H-V Impact	♂	240	2580	No	-	-
18-Jul-04	SNPW322	Vis. Ctr, SNPW	H-V Impact	♀	239	3000	No	-	-
28-Jun-02	PP792	Panther Pk Wash	Suburban	♂	230	1580	Yes	+	-
7-Sep-02	PP131	Panther Pk Wash	Suburban	♂	215	1790	No	-	-
7-Sep-02	PP132	Panther Pk Wash	Suburban	♂	245	2700	No	+	-
7-Sep-02	PP193	Panther Pk Wash	Suburban	♂	199	1270	No	+	+
7-Sep-02	PP194	Panther Pk Wash	Suburban	♀	179	1070	Yes	S	-

Date	Tort ID	Location	Category	Sex	MCL (mm)	Weight (g)	Clinical Signs	ELISA Results	PCR Results
7-Sep-02	PP29	Panther Pk Wash	Suburban	♂	236	2600	Yes	-	-
5-Oct-02	PP410	Panther Pk Wash	Suburban	♀	236	2200	No	-	-
5-Oct-02	PP712	Panther Pk Wash	Suburban	♀	238	2300	No	+	-
2-Aug-03	PP121	Panther Pk Wash	Suburban	♀	235	2320	Yes	+	-
2-Aug-03	PP133	Panther Pk Wash	Suburban	♀	241	2100	No	-	-
2-Aug-03	PP149	Panther Pk Wash	Suburban	♂	191	1200	Yes	-	-
2-Aug-03	PP190	Panther Pk Wash	Suburban	♀	197	1100	No	-	-
2-Aug-03	PP191	Panther Pk Wash	Suburban	♂	272	3350	Yes	+	-
2-Aug-03	PP192	Panther Pk Wash	Suburban	♀	213	1800	No	+	-
2-Aug-03	PP300	Panther Pk Wash	Suburban		180	1060	No	-	-
2-Aug-03	PP408	Panther Pk Wash	Suburban	♀	216	1700	No	-	-
2-Aug-03	PP700	Panther Pk Wash	Suburban	♂	190	1200	Yes	+	-
2-Aug-03	PP802	Panther Pk Wash	Suburban	♂	241	2550	Yes	+	-
2-Aug-03	PP803	Panther Pk Wash	Suburban	♂	219	1940	Yes	+	-
12-Oct-02	RK459	Rocking K	Suburban	♀	240	2340	Yes	+	-
5-Sep-03	RK01	Rocking K	Suburban	♂	246	2550	Yes	+	-
5-Sep-03	RK485	Rocking K	Suburban	♀	233	2200	No	+	-
5-Sep-03	RK565	Rocking K	Suburban	♀	256	3050	Yes	+	-
10-Sep-03	RK103	Rocking K	Suburban	♀	234	2400	No	-	-
10-Sep-03	RK435	Rocking K	Suburban	♂	243	2900	No	+	-
10-Sep-03	RK770	Rocking K	Suburban	♀	240	2550	No	-	-
16-Sep-03	RK113	Rocking K	Suburban	♂	253	2770	No	+	-
16-Sep-03	RK404	Rocking K	Suburban	♂	271	3050	No	+	-
16-Sep-03	RK480	Rocking K	Suburban	♀	244	2550	Yes	+	-
11-Oct-03	RK414	Rocking K	Suburban	♂	240	2550	Yes	+	-
11-Oct-03	RK423	Rocking K	Suburban	♂	245	2650	No	+	-
11-Oct-03	RK555	Rocking K	Suburban	♀	235	2400	Yes	S	-
10-Jul-04	RK481	Rocking K	Suburban	♂	250	2200	No	S	-
10-Jul-04	RK520	Rocking K	Suburban	♀	238	1850	No	+	-
10-Jul-04	RK601	Rocking K	Suburban	♂	231	2000	No	-	N/A
16-Jul-04	RK108	Rocking K	Suburban	♂	218	1300	No	+	N/A
16-Jul-04	RK416	Rocking K	Suburban	♀	222	1600	Yes	+	N/A
13-Sep-03	SR-TM103	Saguaro Ranch	Suburban	♂	217	1700	No	+	-
13-Sep-03	SR-TM105	Saguaro Ranch	Suburban	♂	180	1100	No	-	-
13-Sep-03	SR-TM110	Saguaro Ranch	Suburban	♂	174	900	No	-	-
13-Sep-03	SR-TM118	Saguaro Ranch	Suburban	♀	235	2050	No	+	-
23-Aug-02	TH501	Tumamoc Hill	Suburban	♀	207	1780	Yes	+	-
23-Aug-02	TH508	Tumamoc Hill	Suburban	♂	259	3100	Yes	+	-
14-Sep-02	TH510	Tumamoc Hill	Suburban		134	515	No	-	-
14-Sep-02	TH511	Tumamoc Hill	Suburban	♀	224	2010	No	+	-
20-Sep-03	TH505	Tumamoc Hill	Suburban	♂	244	2600	Yes	+	-
20-Sep-03	TH520	Tumamoc Hill	Suburban	♀	223	2100	Yes	+	-
20-Sep-03	TH521	Tumamoc Hill	Suburban	♂	224	2250	No	-	-
28-Sep-03	TH522	Tumamoc Hill	Suburban	♀	218	2000	No	+	-
27-Sep-03	96H02	Ninetysix Hills	Remote		171	1000	No	-	-
27-Sep-03	96H03	Ninetysix Hills	Remote	♂	219	2310	No	-	-
27-Sep-03	96H04	Ninetysix Hills	Remote	♀	231	2000	No	S	-
27-Sep-03	96H05	Ninetysix Hills	Remote		175	885	No	-	-

Date	Tort ID	Location	Category	Sex	MCL (mm)	Weight (g)	Clinical Signs	ELISA Results	PCR Results
27-Sep-03	96H06	Ninetysix Hills	Remote	♂	237	2300	No	+	-
27-Sep-03	96H07	Ninetysix Hills	Remote	♀	224	2180	No	-	-
27-Sep-03	96H08	Ninetysix Hills	Remote	♂	274	4000	No	+	-
27-Sep-03	96H18	Ninetysix Hills	Remote	♂	251	2250	No	+	-
27-Sep-03	96H19	Ninetysix Hills	Remote	♀	225	2150	No	-	-
27-Sep-03	96H20	Ninetysix Hills	Remote	♂	208	1450	No	+	-
27-Sep-03	96H22	Ninetysix Hills	Remote	♀	240	2400	No	+	-
27-Sep-03	96H23	Ninetysix Hills	Remote	♂	220	2100	No	+	-
27-Sep-03	96H24	Ninetysix Hills	Remote	♀	216	1850	No	+	-
30-Aug-03	BM100	Black Mountain	Remote	♂	232	2150	No	-	-
30-Aug-03	BM101	Black Mountain	Remote	♂	237	1900	No	+	-
30-Aug-03	BM102	Black Mountain	Remote	♂	267	3000	No	-	-
30-Aug-03	BM103	Black Mountain	Remote	♀	240	2400	No	S	-
30-Aug-03	BM104	Black Mountain	Remote	♂	200	1300	Yes	+	-
30-Aug-03	BM117	Black Mountain	Remote	♀	222	2040	No	S	-
30-Aug-03	BM118	Black Mountain	Remote	♂	237	2300	Yes	+	-
30-Aug-03	BM119	Black Mountain	Remote	♀	251	2700	Yes	+	-
30-Aug-03	BM120	Black Mountain	Remote	♂	303	5000	Yes	-	-
6-Sep-03	BM105	Black Mountain	Remote	♂	280	4000	No	+	-
6-Sep-03	BM121	Black Mountain	Remote	♂	274	3350	No	+	-
6-Sep-03	BM122	Black Mountain	Remote	♂	253	3150	Yes	+	-
6-Sep-03	BM123	Black Mountain	Remote	♂	243	2600	Yes	+	-
6-Sep-03	BM124	Black Mountain	Remote	♀	181	1350	No	-	-
6-Sep-03	BM125	Black Mountain	Remote	♂	226	2200	Yes	+	-
6-Sep-03	BM126	Black Mountain	Remote	♀	226	2400	No	+	-
15-Sep-03	DP209	Desert Peak	Remote	♂	211	1700	No	-	-
23-Aug-03	SM800	Stevens Mountain	Remote	♀	229	2150	Yes	-	-
23-Aug-03	SM802	Stevens Mountain	Remote	♂	258	3000	No	+	-
23-Aug-03	SM803	Stevens Mountain	Remote	♂	188	1200	Yes	+	-
31-Aug-03	SM804	Stevens Mountain	Remote	♀	240	2085	No	+	-
31-Aug-03	SM806	Stevens Mountain	Remote	♀	240	2110	No	S	-
31-Aug-03	SM807	Stevens Mountain	Remote	♂	250	2350	No	-	-
14-Sep-03	SM808	Stevens Mountain	Remote	♀	235	2200	No	-	-
14-Sep-03	SM810	Stevens Mountain	Remote	♂	245	2500	Yes	-	-
14-Sep-03	SM817	Stevens Mountain	Remote	♂	270	3150	Yes	-	-
12-Sep-03	SNPECH01	Chimineia Creek	Remote	♂	223	1920	No	+	-
12-Sep-03	SNPECH02	Chimineia Creek	Remote	♀	260	3350	No	-	-
12-Sep-03	SNPECH03	Chimineia Creek	Remote	♂	244	2580	No	-	-
12-Sep-03	SNPECH04	Chimineia Creek	Remote		169	840	No	-	-
13-Sep-03	SNPECH05	Chimineia Creek	Remote	♀	210	1720	No	-	-
13-Sep-03	SNPECH06	Chimineia Creek	Remote	♀	235	2220	No	-	-
13-Sep-03	SNPECH07	Chimineia Creek	Remote	♂	295	4000	Yes	-	-
13-Sep-03	SNPECH08	Chimineia Creek	Remote	♂	255	2550	No	-	-
9-Aug-03	TM801	Derrio Canyon	Remote	♀	152	700	No	-	-
17-Aug-03	TM802	Derrio Canyon	Remote		137	400	No	-	-
17-Aug-03	TM804	Derrio Canyon	Remote		155	695	No	-	-
23-Oct-02	R02185	Tucson	Captive	♂			No	S	-
23-Oct-02	R02187	Tucson	Captive	♂			No	-	-

Date	Tort ID	Location	Category	Sex	MCL (mm)	Weight (g)	Clinical Signs	ELISA Results	PCR Results
23-Oct-02	R02195	Tucson	Captive	♂			No	-	-
23-Oct-02	R02196	Tucson	Captive	♀			No	+	-
23-Oct-02	R02198	Tucson	Captive				No	S	-
27-May-03	TAP0001	Tucson	Captive	♂	250	2800	No	-	-
27-May-03	TAP0002	Marana	Captive	♀	225	1900	No	-	-
27-May-03	TAP0003	Marana	Captive	♂	240	2600	No	-	-
27-May-03	TAP0004	Tucson	Captive	♂	259	3000	Yes	+	-
28-May-03	TAP0005	Tucson	Captive	♂			Yes	+	-
28-May-03	TAP0006	Tucson	Captive	♀			Yes	-	-
28-May-03	TAP0007	Tucson	Captive	♀			Yes	+	-
28-May-03	TAP0008	Tucson	Captive	♀			Yes	+	-
28-May-03	TAP0009	Tucson	Captive	♂			No	+	-
29-May-03	TAP0010	Sahuarita	Captive	♂	216	1600	No	-	-
31-May-03	TAP0011	Tucson	Captive	♂	231	2200	Yes	+	-
1-Jun-03	TAP0013	Tucson	Captive	♀	250	3000	No	-	-
3-Jun-03	TAP0015	Tucson	Captive	♂	200	1400	No	+	-
3-Jun-03	TAP0016	Tucson	Captive	♂	227	1800	No	-	-
3-Jun-03	TAP0017	Tucson	Captive	♂	270	3700	No	+	-
14-Jun-03	TAP0019	Green Valley	Captive	♂	330	6300	No	S	-
14-Jun-03	TAP0020	Green Valley	Captive	♂	280	3850	No	+	-
14-Jun-03	TAP0021	Green Valley	Captive	♂	275	3800	Yes	S	-
27-Jun-03	TAP0022	Catalina Foothills	Captive	♀	253	3000	No	-	-
27-Jun-03	TAP0023	Catalina Foothills	Captive	♀	240	2700	No	+	-
27-Jun-03	TAP0024	Catalina Foothills	Captive	♀	207	2000	No	+	-
2-Jul-03	TAP0025	Catalina	Captive	♂	220	1900	Yes	+	-
2-Jul-03	TAP0026	Catalina	Captive	♂	235	2200	Yes	+	-
2-Jul-03	TAP0027	Oro Valley	Captive	♂	270	3200	No	+	-
3-Jul-03	TAP0028	Tucson	Captive	♂	181	1200	No	S	-
3-Jul-03	TAP0029	Oro Valley	Captive	♂	265	3000	No	-	-
3-Jul-03	TAP0030	Marana	Captive	♀	292	3700	No	-	-
3-Jul-03	TAP0031	Tucson	Captive	♀	233	2300	Yes	+	-
9-Jul-03	TAP0032	Marana	Captive	♂	220	2000	No	-	-
12-Aug-03	TAP0033	Tucson	Captive	♂	235	2200	Yes	+	-
12-Aug-03	TAP0034	Tucson	Captive	♂	245	2700	No	+	-
13-Aug-03	TAP0036	Tucson	Captive	♀	233	2300	No	+	-
13-Aug-03	TAP0038	Tucson	Captive	♂	251	2800	No	-	-
17-Aug-03	TAP0039	Tucson	Captive	♀	232	2700	No	+	-
19-Aug-03	TAP0041	Marana	Captive	♂	240	2300	No	+	-
19-Aug-03	TAP0042	Marana	Captive	♂	200	1300	No	+	-
19-Aug-03	TAP0043	Oro Valley	Captive	♀	247	2800	No	-	-
19-Aug-03	TAP0044	Oro Valley	Captive	♂	301	4900	No	-	-
19-Aug-03	TAP0045	Marana	Captive	♂	247	2900	No	+	-
19-Aug-03	TAP0046	Marana	Captive	♂	258	3000	No	S	-
19-Aug-03	TAP0047	Marana	Captive	♂	275	3300	No	-	-
19-Aug-03	TAP0048	Marana	Captive	♂	261	2600	No	+	-
19-Aug-03	TAP0049	Marana	Captive		145	580	No	S	-
19-Aug-03	TAP0050	Marana	Captive		131	370	No	-	-

Date	Tort ID	Location	Category	Sex	MCL (mm)	Weight (g)	Clinical Signs	ELISA Results	PCR Results
20-Aug-03	TAP0051	Catalina Foothills	Captive	♀	237	2000	No	S	-
20-Aug-03	TAP0052	Catalina Foothills	Captive	♀	260	2800	No	+	-
20-Aug-03	TAP0053	Tucson	Captive	♀	217	2850	No	+	-
20-Aug-03	TAP0054	Tucson	Captive	♀	220	1700	No	-	-
22-Aug-03	TAP0055	Catalina Foothills	Captive	♂	240	2400	No	-	-
22-Aug-03	TAP0056	Catalina Foothills	Captive	♂	260	2850	No	-	-
22-Aug-03	TAP0057	Catalina Foothills	Captive	♂	254	2700	No	-	-
17-Sep-03	TAP0058	Tucson	Captive	♂		1770	No	+	-
29-Oct-03	TAP0059	Tucson	Captive	♂	239	2015	No	-	-
29-Oct-03	TAP0060	Tucson	Captive	♂	275	4000	No	-	-
29-Oct-03	TAP0061	Tucson	Captive	♂			No	-	-
1-Nov-03	TAP0062	Oro Valley	Captive		175	1000	Yes	S	-
2-Nov-03	TAP0064	Tucson	Captive	♂	254	3300	No	+	-
2-Nov-03	TAP0065	Tucson	Captive		150	650	No	S	-
4-Nov-03	TAP0066	Green Valley	Captive	♀	300	4500	No	-	-
4-Nov-03	TAP0067	Sahuarita	Captive	♂	242	2850	No	+	-
4-Nov-03	TAP0068	Sahuarita	Captive	♂	275	4010	No	+	-
6-Nov-03	TAP0070	Tucson	Captive		162	820	No	-	-
8-Nov-03	TAP0072	Tucson	Captive	♀	236	2200	No	+	-
9-Nov-03	TAP0074	Marana	Captive	♂	269	3700	No	S	-

♂ - Male

♀ - Female

+ - Positive

- - Negative

S - Suspect

**APPENDIX C.** Home range size estimates for the MDF and RK sites, Rincon Mountains. 100% MCP, 95% kernel, and 50% kernel home range size estimates for 21 telemetered tortoises at the Mother's Day Fire and Rocking K sites, Rincon Mountains. After accounting for sex and number of observations, ELISA results did not have a significant effect on 100% MCP ( $F_{3,19} = 2.33$ ,  $p = 0.11$ ), 95% kernel ( $F_{3,19} = 0.62$ ,  $p = 0.61$ ), and 50% kernel ( $F_{3,19} = 1.03$ ,  $p = 0.41$ ) home range size estimates.

Tort ID	Sex	# Obs	ELISA	100% MCP (ha)	50% Kernel (ha)	95% Kernel (ha)	Years tracked		Notes
MDF126	♀	46	+	4.530	0.505	3.696	31-Aug-02	23Sep04	Replaced radio
MDF204	♀	48	+	3.788	0.344	3.300	28-Sep-02	26-Aug-04	Removed radio
MDF217	♀	19	+	7.617	1.308	14.274	8-Jun-04	20-Oct-04	NEW
MDF221	♂	29	-	9.001	1.092	11.408	17-Sep-03	19-Oct-04	Replaced radio
MDF298	♀	16	+	0.217	0.032	0.264	28-Sep-02	31-May-03	Missing
MDF300	♀	12	-	5.992	1.165	9.872	26-Aug-04	21-Oct-04	NEW
MDF339	♂	60	+	7.790	0.256	1.980	23-Mar-02	26-Jul-04	Removed radio
MDF390	♂	33	+	2.404	0.257	1.588	23-Mar-02	21-Jun-04	Removed radio
MDF410	♀	75	+	8.814	0.341	2.669	23-Mar-02	2-Sep-04	Replaced radio
MDF422	♂	56	+	13.298	0.582	6.159	23-Mar-02	19-Oct-04	Replaced radio
MDF712	♂	66	+	20.152	1.508	12.204	23-Mar-02	2-Sep-04	Replaced radio
MDF721	♂	35	+	2.308	0.253	1.624	30-May-02	13-Nov-03	Found dead
RK103	♀	89	-	8.387	1.330	7.020	5-Mar-02	11-Nov-04	Replaced radio
RK404	♂	77	+	16.330	0.532	2.836	10-May-02	11-Nov-04	Replaced radio
RK414	♂	76	+	13.362	4.040	14.364	29-Mar-02	11-Nov-04	Replaced radio
RK435	♂	49	+	8.964	0.446	5.000	29-Mar-02	29-May-03	Removed radio
RK459	♀	30	+	8.964	0.446	5.000	28-Aug-02	15-Jul-03	Missing
RK480	♀	59	+	20.063	2.204	13.735	29-Mar-02	2-Mar-04	Removed radio
RK485	♀	87	+	7.899	0.375	3.126	1-Mar-02	30-Aug-04	Removed radio
RK565*	♀	77	+	12.075	2.328	13.557	1-Mar-02	23-Jul-04	Missing
RK770	♀	62	-	13.329	0.917	8.172	25-Jun-02	10-Jul-04	Removed radio
MDF711▪	♀	24	S	53.399	6.117	63.442	31-Aug-02	10-May-03	Missing
MDF722▪	♀	29	S	4.899	0.970	7.997	9-Oct-03	19-Oct-04	Replaced radio
RK481▪	♂	78	S	24.172	2.580	17.362	1-Mar-02	30-Aug-04	Removed radio
RK555▪	♀	36	S	41.212	10.640	60.466	22-Jun-02	11-Oct-03	Removed radio

\* 4 location observations excluded from home range analyses

▪ ELISA suspect, excluded from home range analyses

♂ Male

♀ Female

+ Positive

- Negative

S Suspect

**APPENDIX D.** Press release issued on 09 August 2004 providing information on desert tortoise movements, encounters, natural history, protection and captivity.



**National Park Service**

Saguaro National Park

3693 South Old

Spanish Trail

**U.S. Department of the Interior**

Tucson, AZ 85730

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## Saguaro National Park News Release

*For Immediate Release*

August 9, 2004

Contact: Don Swann, (520) 733-5177

### **Give a Hand to Desert Tortoises**

With the arrival of the summer monsoons, desert tortoises are entering their most active season. Unfortunately, increasing contact between wild tortoises and people in the Tucson area brings increased risks to these slow-moving symbols of the Sonoran Desert. University of Arizona, Saguaro National Park, the Arizona Game and Fish Department, the Arizona-Sonora Desert Museum and others have teamed up to spread the word on the best ways that all of us can help protect tortoises in the wild.

Should you encounter a wild desert tortoise, please **do not** remove it from its habitat. “People think that they are doing the animals a favor by taking them home,” said Don Swann, a wildlife biologist with Saguaro National Park, “but it is usually harmful to the tortoise.” Taking a wild tortoise home is not only illegal in Arizona, but the animal will die if it is not well cared-for. Most tortoises stay in the same small area their entire lives, so moving one to a new location is considered risky because the tortoise will likely not know where to find food and shelter.

If you do come across a desert tortoise crossing a busy road, you can be of great help. After keeping traffic safety in mind, experts recommend picking the tortoise up and moving it, gently, to the other side of the road. Carry it so that it is level to the ground, and move it in the same direction it was headed.

In contrast, if you have a desert tortoise that you have been keeping as a pet at home, please **do not** release it into the wild. Biologists are particularly concerned with the occurrence of Upper Respiratory Tract Disease (URTD), which is associated with significant declines in tortoises in the Mojave Desert, and may be caused by infected captive tortoises coming in contact with wild tortoises.

If you want to share your yard with a desert tortoise, they can be lawfully obtained through state-sanctioned adoption facilities such as Adobe Mountain Rehabilitation Center (Phoenix) or the Arizona-Sonora Desert Museum (Tucson).

Additional background:

Desert tortoises are estimated to live as long as 50 to 100 years. Adults are generally about 10-14 inches in length. Desert tortoises are most common in the rocky foothills surrounding Tucson, but these areas are also popular places for people to live and are being rapidly developed. While the desert tortoise has been named a Threatened Species under the U.S. Endangered Species Act in areas of California, Nevada, Utah and Northern Arizona, tortoise populations are healthier in southern Arizona and the tortoise is not threatened here. However, the species is protected throughout Arizona.

Desert tortoises in Arizona are considered a species of special concern and Commission Order 43 prohibits taking desert tortoises from the wild. The Arizona Game and Fish Department also list desert tortoises as Restricted Live Wildlife, which means they cannot be imported into or exported from the state. Federal law also precludes transportation of desert tortoises across state lines.

Other contacts:

Roy Averril-Murray, State Herpetologist, Arizona Game and Fish Department,  
[rmurray@gf.state.az.us](mailto:rmurray@gf.state.az.us), (602) 789-3505

Craig Ivanyi, Curator of Herpetology, Ichthyology, & Invertebrate Zoology, Arizona-Sonora Desert Museum, [civanyi@desertmuseum.org](mailto:civanyi@desertmuseum.org), (520) 883-2702

James L. Jarchow, Veterinarian, Orange Grove Animal Hospital, [jjarchow@svg-vets.com](mailto:jjarchow@svg-vets.com), (520) 877-2626

Cristina Jones, Graduate Research Assistant (studying URTD), University of Arizona,  
[cajones@u.arizona.edu](mailto:cajones@u.arizona.edu), (520) 471-4278

Stéphane Poulin, Keeper, Department of Herpetology, Ichthyology, & Invertebrate Zoology, Arizona-Sonora Desert Museum, [spoulin@desertmuseum.org](mailto:spoulin@desertmuseum.org), (520) 883-2702

Cecil Schwalbe, Research Ecologist, US Geological Survey, [cecils@ag.arizona.edu](mailto:cecils@ag.arizona.edu), (520) 621-5508

Cyndy Wicker, Tortoise Adoption Program Coordinator (volunteer), (520) 883-3062

**Appendix D1.** *Arizona Daily Star* article prompted by press release.***Neighborhood Briefing****Arizona Daily Star, The (Tucson, AZ)**August 16, 2004***Learn to protect wild *desert tortoises***

Saguaro National Park, the University of Arizona, the Arizona Game and Fish Department, the Arizona-Sonora **Desert** Museum and others have teamed up to spread the word on the best ways that all of us can help protect **tortoises** in the wild.

Should you encounter a wild **desert tortoise**, do not remove it from its habitat. "People think that they are doing the animals a favor by taking them home," Don Swann, a wildlife biologist with Saguaro National Park, said in a press release. "But it is usually harmful to the **tortoise**." Taking a wild **tortoise** home is illegal in Arizona.

If you do come across a **desert tortoise** crossing a busy road - keeping traffic safety in mind - experts recommend picking the **tortoise** up and moving it, gently, to the other side of the road. Carry it so that it is level to the ground, and move it in the same direction it was headed.

If you have a **desert tortoise** that you have been keeping as a pet, do not release it into the wild. Biologists are particularly concerned with the occurrence of upper respiratory tract disease, which may be caused by infected captive **tortoises** coming into contact with wild **tortoises**.

If you want to share your yard with a **desert tortoise**, they can be lawfully obtained through state-sanctioned adoption facilities such as the Arizona-Sonora **Desert** Museum.

\* Compiled by Angela Soto. Call 573-4142 or e-mail [asoto@azstarnet.com](mailto:asoto@azstarnet.com).

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**Appendix D2.** *Desert Times* article prompted by press release.**Give a Hand to Desert Tortoises**

With the arrival of the summer monsoons, desert tortoises are entering their most active season. Unfortunately, increasing contact between wild tortoises and people in the Tucson area brings increased risks to these slow-moving symbols of the Sonoran Desert.

Should you encounter a wild desert tortoise, please do not remove it from its habitat. Taking a wild tortoise home is not only illegal in Arizona, but the animal will die if it is not well cared for. Most tortoises stay in the same small area their entire lives, so moving one to a new location is considered risky because the tortoise will likely not know where to find food and shelter.

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If you want to share your yard with a desert tortoise, they can be lawfully obtained through state sanctioned adoption facilities such as the Arizona Sonora Desert Museum.

Desert tortoises are estimated to live as long as 50 to 100 years. Adults are generally about 10-14 inches in length. Desert tortoises are most common in the rocky foothills surrounding Tucson, but these areas are also popular places for people to live and are being rapidly developed. While the desert tortoise has been named a Threatened Species under the U.S. Endangered Species Act in areas of California, Nevada, Utah and Northern Arizona, tortoise populations are healthier in southern Arizona and the tortoise is not threatened here. However, the species is protected throughout Arizona.

Desert tortoises in Arizona are considered a species of special concern and Commission Order 43 prohibits taking desert tortoises from the wild. The Arizona Game and Fish Department also lists desert tortoises as Restricted Live Wildlife, which means they cannot be imported into or exported from the state. Federal law also precludes transportation of desert tortoises across state lines.

•Craig Ivanyi, Curator of Herpetology, ASDM, [civanyi@desertmuseum.org](mailto:civanyi@desertmuseum.org), 883-2702

•Cristina Jones, Graduate Research Assistant (studying URTD), U of A, [cajones@u.arizona.edu](mailto:cajones@u.arizona.edu), 471-4278.

**APPENDIX E. Dates, Presenters, Locations, and Audience of Public Outreach.**

Date	Presenter	Location	Audience
3-Jun-03	Don E. Swann	Presented research and information about Desert Tortoises at the Junior Ranger Camp at Saguaro National Park East.	20 Children ages 6-11
10-Jun-03	Don E. Swann	Presented research and information about Desert Tortoises at the Junior Ranger Camp at Saguaro National Park West.	20 Children ages 6-11
1-Feb-04	Cristina Jones	Presented research to Arizona-Sonora Desert Museum Tortoise Adoption Program (TAP) Staff and Volunteers.	3 ASDM Staff, 8 TAP volunteers
31-Mar-04	Don E. Swann	Presented research and information about Desert Tortoises to Conservation Biology Internship class at University of Arizona.	12 Univ. of AZ students
13-May-04	Cristina Jones	Presented poster at Biodiversity and Management of the Madrean Archipelago II, 5th Conference on Research and Resource Management in the Southwestern Deserts. Double Tree Hotel Tucson at Reid Park.	25 Adults
19-May-04	Taylor Edwards	Public presentation on Desert Tortoises at the International Wildlife Museum.	20 Adults, 5 Children
8-Jun-04	Don E. Swann	Presented research and information about Desert Tortoises at the Junior Ranger Camp at Saguaro National Park East.	20 Children ages 6-11
15-Jun-04	Don E. Swann	Presented research and information about Desert Tortoises at the Junior Ranger Camp at Saguaro National Park East.	20 Children ages 6-11
16-Jun-04	Cristina Jones	Presented poster at the AIDTT's open house at the Phoenix Zoo.	10-15 Adults
2-Sep-04	Taylor Edwards	Presented information on desert tortoises to the Reid Park Zoo Docents.	35 Adults
4-Oct-04	Taylor Edwards	Presented information about Desert Tortoises to the Notch Neighborhood Association, adjacent to Saguaro National Park East.	30 Adults
15 - 17 October 2004	Cristina Jones	Presented research and information about Desert Tortoises to the public at the AZ PARC Education Outreach Technical Working Group Information booth at the SAHBA (Southern Arizona Home Builders Association) Fall Home and Garden Show.	Booth visited by 1,500 people / day, ~200 adults & 60 children asked about tortoise in 3days.
21 - 22 Feb04	Cristina Jones	Poster Presentation at the 29th Annual Desert Tortoise Council Symposium. Sam's Town Hotel and Casino, Las Vegas, NV.	25 Adults

**APPENDIX F.** So, you've found a desert tortoise... Now what? Brochure, page one.  
 Brochure available as a PDF on the Rincon Institute website:  
<http://www.rinconinstitute.org/deserttortoise.pdf>

**There are many websites that have information on Desert Tortoises in Arizona. We recommend:**

Arizona Game and Fish Department:  
[azgfd.gov/w\\_c/desert\\_tortoise.shtml](http://azgfd.gov/w_c/desert_tortoise.shtml)  
 Arizona Game and Fish Department's  
 Wildlife Rehabilitation Center in Phoenix  
[www.wildlifeaux.org/tortoise/tortoise.htm](http://www.wildlifeaux.org/tortoise/tortoise.htm)  
 Arizona-Sonora Desert Museum:  
[www.desertmuseum.org/programs/tap.html](http://www.desertmuseum.org/programs/tap.html)  
 Saguaro National Park:  
[nps.gov/sagu/research/index.htm](http://nps.gov/sagu/research/index.htm)  
 Tucson Herpetological Society:  
[tucsonherpsociety.org/](http://tucsonherpsociety.org/)  
 Arizona PARC  
[www.reptilesfaz.com/Turtle-Amphibiansubpages/h-g-agassizii.html](http://www.reptilesfaz.com/Turtle-Amphibiansubpages/h-g-agassizii.html)



**How can I adopt a desert tortoise?**

Although it is illegal to remove a desert tortoise from the wild, you can legally adopt a desert tortoise from a state-sanctioned adoption program such as Arizona Game and Fish Department's Wildlife Rehabilitation Center in Phoenix, and the Arizona-Sonora Desert Museum (Tucson) or by contacting your local Arizona Game and Fish Department office.

**Arizona Game and Fish Department contact information:**

Phoenix  
 2221 W. Greenway Rd.  
 Phoenix, AZ 85023-4399 602-942-3000

Arizona Game and Fish Department's Wildlife Rehabilitation Center in Phoenix 623-582-9806

Region III – Kingman  
 5325 N. Stockton Hill Rd.  
 Kingman, AZ 86401 928-692-7700

Region IV - Yuma  
 9140 E. 28th St.  
 Yuma, AZ 85365 928-342-0091

Region V - Tucson  
 555 N. Greasewood Rd.  
 Tucson, AZ 85745 520-628-5376

**So, you've found a desert tortoise...**



**Now what?**

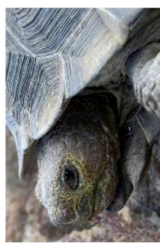


**Heritage Fund**

Brochure written in fulfillment of the Arizona Game and Fish Department Heritage Grant "Mycoplasma agassizii in Desert Tortoises" Project#U03005 with additional support from:



January 2005

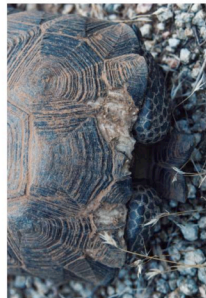
**APPENDIX F Continued.** So, you've found a desert tortoise... Now what? Brochure, page two.

Should you encounter a desert tortoise near a natural, relatively undeveloped area, it is most likely a wild

desert tortoise. Enjoy this unique natural experience, but **do not disturb the tortoise**. Desert tortoises are well adapted to living in an often harsh desert environment, and know where to find food and shelter. Taking a wild tortoise home is not only illegal in Arizona, but also harmful to the tortoise as the animal will die if not well cared-for. Most tortoises stay in the same small area their entire lives, so moving one to a new location can threaten the tortoise's survival. The best way to help a wild tortoise is to leave it alone.

The only time that it is okay to touch a wild desert tortoise is if you find it crossing a busy road. Keeping traffic safety in mind, pick the tortoise up and move it, gently, to the other side of the road. Carry it so that it is level to the ground, and move it in the same direction it was headed.

If you are within a metropolitan or developed area, away from natural desert or the edge of town, you have most likely found a captive desert tortoise. Some people keep adopted desert tortoises as pets which may exhibit obvious signs of captivity such as paint or holes drilled in the shell. It is essential to keep captive tortoises separate from wild tortoises because captive tortoises may carry diseases. If you have encountered a captive tortoise, contact your local Arizona Game and Fish Department office.

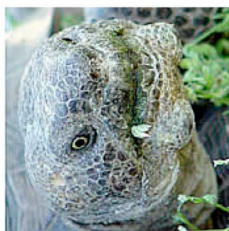


### What are the main threats to desert tortoises?

Threats to desert tortoises include:

- diseases • habitat loss • illegal collection
  - attacks by domestic and feral dogs • roads
- Due to these threats, desert tortoises are considered to be a species of special concern in Arizona.

### How do desert tortoises survive in the desert?



Desert tortoises spend much of their lives underground in burrows or caves that provide protection from extreme temperatures and predators. Tortoises drink rain water that collects in pools, and also obtain some water from the plants they eat.

### Why shouldn't I pick up a desert tortoise?

When frightened, a desert tortoise may empty its bladder as a defense mechanism. Tortoises store water in their bladders and reabsorb it in dry times of the year. Loss of bladder fluids can cause dehydration and death.

### What can I do to help conserve desert tortoises?

If you find a desert tortoise in the wild, do not handle it; instead, enjoy it at a distance. Drive slowly on desert roads to avoid accidentally injuring or killing tortoises. You can participate in the Arizona Game and Fish Department's Sponsor-a-Tortoise Program by making a donation to the Desert Tortoise Project. Contributions provide support for research supplies and radio transmitters used to learn about and monitor desert tortoises. Domestic dogs have been known to maim and even kill desert tortoises, so please do not allow uncontrolled dogs out of your yard or vehicle.

You can report any harassment or illegal collecting of desert tortoises by calling the Arizona Game and Fish Department's Operation Game Thief at 1-800-352-0700.

Spread the word! Together, we can help the desert tortoise survive.

### What do I do if I have a tortoise and can no longer care for it?



If you have a desert tortoise that you have been keeping as a pet, do not release it into

the wild. Biologists are concerned with the occurrence of Upper Respiratory Tract Disease (URTD), which is associated with significant declines in tortoises in the Mojave